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SURVIVAL OF PARASITE EGGS IN STORED SLUDGE

by

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16. ABSTRACT

The inactivation rates of digester-resistant parasite eggs in laboratory-stored sludge were measured to determine their potential fate in sludge lagoons. Eggs from roundworms (Ascaris, Toxocara and Trichuris) and a tapeworm (Hymenolepis) were added to domestic sludges either before and during, or after aerobic or anaerobic digestion. Digested sludge samples seeded with the parasite eggs were stored in the laboratory at 4°C, 25°C, and in a container that was inserted in the ground to simulate sludge storage conditions. Non-sludge soil samples (controls) were seeded with the same parasites as the digested sludges and stored under similar conditions.

The total number of eggs recovered from the samples decreased as storage time and temperature increased. The number of viable eggs and the potential infectivity of recovered Toxocara and Ascaris eggs were related primarily to storage temperature and time. After 25 months of storage at 4°C, the Toxocara eggs and some Ascaris eggs remained both viable and infective, whereas most of the eggs stored at 25°C were nonviable after 10 to 16 months of storage in sludge. Though storage temperature and storage time were the most important factors in the inactivation of these eggs, minor effects were also associated with other factors--type of sludge digestion, timing of egg addition, type of storage (in sludges versus soil), pH, and species of egg. These controlled laboratory studies suggest that sludge lagooning can be an effective method for eliminating parasite eggs, particularly in warmer geographic locations.

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FOREWORD

The U.S. Environmental Protection Agency is charged by Congress with protecting the Nation's land, air, and water systems. Under a mandate of national environmental laws, the agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. The Clean Water Act, the Safe Drinking Water Act, and the Toxic Substances Control Act are three of the major congressional laws that provide the framework for restoring and maintaining the integrity of our Nation's water, for preserving and enhancing the water we drink, and for protecting the environment from toxic substances. These laws direct the EPA to perform research to define our environmental problems, measure the impacts, and search for solutions.

The Water Engineering Research Laboratory is that component of EPA'S Research snd Development program concerned with preventing, treating, and managing municipal and industrial wastewater discharges; establishing practices to control and remove contaminants from drinking water and to prevent its deterioration during storage and distribution; and assessing the nature and controllability of releases of toxic substances to the air, water, and land from manufacturing processes and subsequent product uses. This publication is one of the products of that research and provides a vital communication link between the researcher and the user community.

A steady growth in urban areas brings with it the need to dispose of increased products of municipal sewage treatment. Land application of sewage sludge is an attractive solution, but this practice should not endanger either human beings or domestic animals. This research study has measured the inactivation of several parasite eggs during long-term sludge storage at various temperatures.

Francis T. Mayo
Director
Water Engineering Research Laboratory

ABSTRACT

The inactivation rates of digester resistant parasite eggs in laboratory-stored sludge were measured to determine their potential fate in sludge lagoons. Eggs from roundworms (*Ascaris*, *Toxocara*, and *Trichuris*) and a tapeworm (*Hymenolepis*) were added to domestic sludges either before and during, or after aerobic or anaerobic digestion. Digested sludge samples seeded with the parasite eggs were stored in the laboratory at 4°C, at 25°C, and in a container that was inserted in the ground to simulate sludge storage conditions. Non-sludge soil samples (controls) were seeded with the same parasites as the digested sludges and stored under similar conditions.

The total number of eggs recovered from the samples decreased as storage time and temperature increased. The number of viable eggs and the potential infectivity of recovered *Toxocara* and *Ascaris* eggs were related primarily to storage temperature and time. After 25 months of storage at 4°C, the *Toxocara* eggs and some *Ascaris* eggs remained both viable and infective, whereas most of the eggs stored at 25°C were non-viable after 10 to 16 months of storage in sludge. Though storage temperature and time were the most important factors in the inactivation of these eggs, minor effects were also associated with other factors--type of sludge digestion, timing of egg addition, type of storage (in sludges versus soil), pH, and species of egg. These controlled laboratory studies suggest that sludge lagooning can be an effective method for eliminating parasite eggs, particularly in warmer geographic locations.

This report was submitted in fulfillment of Cooperative Agreement No. CR806954-02 by the University of Cincinnati under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period September 10, 1979, to September 10, 1983, and the work was completed as of November 1984.

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SECTION 1

INTRODUCTION

BACKGROUND

The use of treated (digested or stabilized) sewage sludges as a fertilizer to replenish the nutrients in agricultural or forage soils is considered an alternative to either dumping, burying or incinerating the sludge. Sewage sludge also is used to reclaim strip-mined soils or is disposed of on dedicated land where crops may be grown but often are not. In the United States, approximately one-third of the sewage sludge generated is applied to the soil. In states such as in Ohio, approximately 60% of the sewage sludge generated is applied to the land (1).

Sewage sludges are the by-products of sewage treatment. The major residue streams produced are raw primary sludge (from primary settling) and waste-activated sludge (WAS) from biological treatments of primary effluent, or in some cases from direct treatment of the raw wastewater. A major portion of the raw primary sludge is composed of human fecal wastes and contains pathogenic organisms (viruses, bacteria, protozoa, and helminth eggs). Waste-activated sludge contains pathogens that have escaped the primary settling process and bacteria and other microbial forms that have multiplied in the biological treatment of the wastewater.

Undigested or unstabilized municipal sewage sludges are usually treated by conventional sludge treatment processes. The most common sludge treatment process is digestion, usually conducted under anaerobic treatment conditions. Conventional sewage sludge digestion treatment under ambient or mesophilic treatment conditions removes most but not all of the pathogens that can be harmful to man and animals. Parasites are of concern because of their particular resistance to conventional sludge treatments.

No evidence exists that proper land spreading of digested or otherwise conventionally stabilized sludges has caused disease in man or animals. Nevertheless, concern exists that the pathogens left in digested sludges may contribute to human and animal diseases. Survival studies of pathogens in stabilized sludges indicate that the parasites, particularly the digester-resistant roundworms, will survive the longest in soils under most environmental conditions. Therefore, once these sludges are disposed of on the land, the viable parasite eggs that have survived digestion may develop into an infective stage and pose a potential source of infection to humans and animals (2). Studies have also shown that

aerobic digestion of agricultural wastes causes Ascaris eggs to embryonate rapidly (but not hatch) (3). Applying embryonated eggs to soils greatly enhances the risk of infection. Ascaris and Toxocara eggs were recovered by Theis (4) when he examined soil upon which sludge had been deposited. Eggs were also found in the irrigation return flows from land to which sludges had been applied. Fitzgerald (5) exposed pathogen-free swine to anaerobically digested sludges containing Ascaris eggs through sludge application to soil or directly to the swine. Some of the eggs were ingested by the animals and eventually caused light infestation in their gastrointestinal tracts.

The concern, therefore, of parasite survival in soils stems from the potential human and animal health threat that can occur from Ascaris, Toxocara or other digester-resistant parasites (e.g. roundworms). Ascaris can migrate through the liver and lungs and can cause certain forms of pneumonia in humans and in animals. Visceral larva migrans can be a source of infection from Toxocara, and cutaneous larvae migrans can be a source of hookworms from domestic animals. Population growth, improved wastewater treatment that produces more sludge, and greater interest in the value of sludge as a fertilizer have all increased the potential of contact of humans and animals to sewage sludge used or disposed of on or in the soil.

LONG-TERM STORAGE AS A PROCESS TO INACTIVATE PARASITE EGGS

Under the requirements of the Resource Conservation and Recovery Act (RCRA), the U.S. Environmental Protection Agency placed in the Federal Register (Volume 44, No. 179, Thursday, September 13, 1979) Interim Final, and Proposed Regulation (Criteria), 40 CFR Part 257 "Criteria for Classification of Solid Waste Disposal Facilities and Practices." Under these Criteria, sludge is defined as a solid waste. Also, under the Criteria, untreated or raw sewage sludge cannot be applied to, incorporated into, or injected shallowly into the land surface. Appendix II of the Criteria defines the processes and the process conditions for acceptable treatment of sewage sludge for pathogen reduction before use on land. Appendix II A defines "Processes to Significantly Reduce Pathogens". These processes include ambient and mesophilic aerobic digestion, ambient, mesophilic, and low-temperature thermophilic anaerobic digestion, mesophilic temperature sludge composting; lime stabilization and air drying. The basis behind these processes is that pathogenic bacteria and enteric viruses will be reduced by at least 1 log over that of untreated sewage sludge, similar to good anaerobic digestion. In addition, odor and vector conditions are also substantively reduced or eliminated before soil application. Because pathogenic organisms can survive these processes, certain restrictions are placed on use of the sludge applied to soil for growing of crops and for human and animal contacts. These restrictions are as follows: (1) crops grown for human consumption that can contact the sludge cannot be grown within 18 months of sludge application or incorporation. This 18-month restriction is not required if the edible portions of the crops do not contact the sludge; (2) public access is controlled for at least 12 months; and (3) grazing by animals whose products are consumed by humans is avoided for at least 1 month after sludge application.

Appendix II B of the Criteria defines the acceptable "Processes to Further Reduce Pathogens." These processes use higher temperatures and/or irradiation (e.g., gamma rays or high-energy electron irradiation) to disinfect the sludge to a level that would result in pasteurization treatment at 70°C for at least 30 minutes. Sludges whose pathogen contents have been further reduced are not required to meet any other disease requirement of the Criteria, and the sludge can be applied without the pathogen restrictions cited in the above paragraph. Examples of "Processes to Further Reduce Pathogens" are thermophilic (above 55°C) composting, thermophilic aerobic digestion, heat drying, heat treatment and for irradiation - high energy electrons or gamma rays. These pathogen disinfection processes are relatively expensive and can require the use of additional heat energy to achieve the higher thermophilic temperatures. For either "A" or "B" pathogen reduction processes, other methods may be acceptable if the pathogen densities are reduced to equivalent levels.

A sludge treatment process notably absent from the appendix of the Criteria is long-term storage of sludge (sludge lagooning). This sludge treatment process is frequently used by many publicly owned treatment works (POTW's) throughout the country as an intermediate step before disposal (usually) on the land, or as a final disposal method in itself. This process is relatively easy to operate. Sludge lagooning is known to have the following advantages: (1) concentrating the sludge solids, (2) providing storage capacity for digested sludge, (3) providing additional stabilization, and (4) reducing pathogen levels (a side benefit of sludge storage in lagoons that has been recognized for years)(6,7). To avoid noxious odors and for vector control, sewage sludge must be stabilized before storage.

Because very few systematic studies have been conducted, data are very sparse on the effectiveness of sludge lagooning for reducing pathogen levels. Hinesley and others have reported a 99.9-percent reduction in fecal coliforms after about 30 days of storage (8). A 24-week laboratory sludge (anaerobically digested) storage study by Stern and Farrell (6) showed that substantial density reductions in indicator bacteria, Salmonella sp., and enteric virus will occur within 60 days at 20°C (68°F), whereas even after 24 weeks of storage at 4°C (35°F), indicator bacteria organisms and enteric virus were never below the detectable limits and just reached below detectable limits for Salmonella sp. Qualitative measurements indicated that Ascaris suum survived the 24-week sludge storage test period. Storm and others (9) showed fecal coliform reductions of one to three orders of magnitude from long-term storage of an anaerobically digested mixture of primary and waste-activated sludge in facultative lagoons.

The above studies strongly suggest that long-term storage of sludge for at least 6 months will inactivate indicator bacteria, pathogenic bacteria and enteric viruses, particularly in warm environments. Parasites, however, will survive. There are some other data that suggest the possibility that longer storage of sludge could also inactivate parasites. Schatzle (10) reported a 10-percent reduction of Ascaris egg viability after 7 months storage at 0 to 20°C. Veerannan (11) reported a 50-percent reduction in Ascaris egg viability after 1-year storage and

practically no survival after 3 years.

Thus, there is evidence to suggest that digester resistant parasites in sewage sludges could be inactivated by long-term storage, probably under warmer temperature environments. If this inactivation could be achieved, together with the known inactivation that occurs for indicator bacteria, pathogenic bacteria and enteric viruses, then long-term storage could be a viable, relatively low cost sludge treatment alternative (together with a prior sludge stabilization process) to achieve the same degree of total disinfection as defined for "Processes to Further Reduce Pathogens" under Appendix II B of the Criteria. Further, long-term sludge storage also could be of beneficial health benefit in areas of the world, particularly in developing countries, where parasite infectivity is endemic from sludges.

OBJECTIVE OF THIS STUDY

It was the basic objective of this laboratory study to determine whether digester-resistant helminths (roundworms) in anaerobically or aerobically digested sludges could be inactivated by long-term storage under proper temperature environments. Because of the generally low levels of parasites in sludges, it was necessary to add parasites to the digested sludges so that inactivation curves could be developed with time. The parasites added to the digested sludges (either after digestion or prior to and during digestion) were taken from feces or intestines of infected animals to insure that the most resistant forms, as normally observed in the environment, were tested in this inactivation study.

SECTION 2

CONCLUSIONS

1. During the 15-day period of mesophilic anaerobic digestion approximately 23% of the Ascaris suum eggs were inactivated; Toxocara canis and Trichuris spp. eggs were not affected.

2. Aerobic digestion inactivated 38% of the Ascaris eggs, 11% of the Trichuris eggs but none of the Toxocara eggs.

3. The total number of eggs recovered from the stored samples decreased with time.

4. Eggs of all the species studied were mostly inactivated faster (16 months in anaerobic digested sludges a about 25 months in aerobic digested sludges) when stored at higher (25°C) rather than at lower temperatures (4°C or in the ground).

5. Ascaris eggs aerobically digested along with sludge (Phase II) were noninfective after 25 months when stored at 25°C, in the ground, and at 4°C.

6. Ascaris eggs anaerobically digested with sludge (Phase II) were noninfective by 16 months when stored at 25°C but were still infective after 22 months when stored at 4°C or in the ground.

7. Either aerobic or anaerobic digestion makes Toxocara canis eggs more susceptible to inactivation when compared with eggs stored in soil controls.

8. Trichuris spp. eggs in the digested sludges (Phase II) appeared to be inactivated by storage at 25°C and in the ground after 16 months but could be viable even after 25 months when stored at 4°C. However, a conclusive statement on Trichuris viability is difficult to make because of the low initial innoculum levels and subsequent low recovery of these eggs.

9. Hymenolepis diminuata proved very sensitive, since storage of the eggs at 4°C alone before seeding into the sludges rendered them nonviable and noninfective.

10. Viability of the eggs did not always predict infectivity; that is, eggs that did not look viable were sometimes infective and vice versa.

SECTION 3

RECOMMENDATIONS

This 3-year investigation into the persistence of certain roundworm eggs after anaerobic or aerobic digestion with sludge indicates that additional information is needed in the following specific areas:

1) Investigation of the types and numbers of microbial agents present in stored sludges and their contributions to the destruction of parasite eggs.

2) Investigation of the factors in the digestion processes that result in an apparent increased resistance of Ascaris suum eggs to inactivation when they are subsequently placed into storage.

3) A thorough measurement of the redox potential of sludges during storage and the relation of the electrochemical potentials to the organic and inorganic chemical reactions that may affect parasite destruction.

4) A larger-scale investigation into the effects of long-term storage on the destruction of eggs of certain parasites that more closely duplicates existing lagoon conditions.

5) Information on inactivation of parasite eggs within 3 months of storage in the absence of retrieving them from either sludge or soil to more conclusively establish whether they were inactivated or whether they became more difficult to isolate.

6) Studies on die-off rates in sludge-amended soils.

7) Biochemical and physiological analyses of parasite eggs to determine what is altered in eggs exposed to sludge compared with soil or water controls.

8) Research to improve the analytical methods for measuring the recovery and viability of parasite eggs in sludges (e.g. improve the standard deviation of the method).

SECTION 4

METHODS AND MATERIALS

OVERVIEW

The basic objective of this laboratory study was to measure the inactivation rates of specific digester resistant parasites in digested sewage sludges stored under specified conditions. Digester resistant eggs of the nematodes Ascaris suum, Toxocara canis, and Trichuris spp., and a cestode-- Hymenolepis diminuta, were seeded into sludge before and during, or after anaerobic or aerobic digestion. The purpose of the addition of large numbers of the parasite eggs was to ensure sufficient numbers in the digested sludges for developing inactivation curves with storage time and also to increase the statistical significance of the numbers (or absence) of viable surviving eggs.

The parasite eggs added to the digested sludges were taken from the feces or intestines of infected hosts. This insured that normal resistant stages of the test parasites were used for meaningful test results for estimations of what would be expected to occur under normal sludge lagoon storage conditions. Studies by Reimers et al. (12) have shown a significant increase in the resistance of the parasites to sludge inactivation treatments when the eggs are taken from the infected host as compared to directly extracting the parasite eggs from the female worm.

Inactivation rates of the test parasites in digested sludges were measured under two constant storage temperatures--25°C and 4°C. To simulate actual sludge storage lagoon effects, samples of the parasite-spiked digested sludges were placed in 55-gallon drums that were then inserted into the ground with the covers of the drums flush with the ground's surface thus exposing the test samples to the normal temperature fluctuations that occur in a temperate climate. Test samples were taken at the beginning of the storage period and every 3 months thereafter for up to about 24 months, and then, after 30 months and 33 months for a select number of test samples for additional information. Initially, the protocol was to measure the number of parasites recovered and the viability of the recovered parasites as an indication of potential infectivity. Three months after the sludge storage sampling began, actual infectivity studies were initiated using the Ascaris suum and Toxocara canis recovered from the test samples with rats as the indicating host. Trichuris eggs were recovered in numbers too low to be used in infectivity studies. Adding the infectivity studies greatly increased the usefulness of the results of the study.

The literature indicates that apparently little information is available for conducting this type of parasite study with sewage sludges. It was felt that a control was needed for comparison of the parasite inactivation effects in digested sewage sludges. There is little literature information available to suggest the type or types of controls to use. It was arbitrarily chosen to use a pseudo-soil control at a solids concentration equivalent to the solids concentrations in the digested sludge samples. These soil control samples were also spiked with approximately the same levels of the three different nematode and the cestode eggs as in the digested sludge samples and stored under the same test conditions. The same analyses for parasite recovery, viability and infectivity were conducted for these control samples. On hindsight (and if funds were available), it would probably have been of benefit to use as a control the same levels of parasites in distilled water, physiological saline, or in an otherwise empty container under the same test conditions. It was felt, however, at the start of the study that the samples (both sludges and the pseudo-soil controls) stored at 4°C could also be considered as control samples since little inactivation of the parasites was expected at this low temperature. Also, recovery tests of parasites spiked in the digested sludges showed that the recovery of parasites with the method used for this study (to be discussed in a subsequent part of this section) would be approximately 78 percent. As it turned out, the inactivation of Ascaris suum eggs was nearly the same in the soil controls as in the digested sludges under the same storage conditions. Also, unexpectedly, there was a rapid decrease in parasite recovery from the digested sludge samples and pseudo-soil control samples after only 3 months of storage. While it is believed that this rapid decrease in the parasite recovery is due to the initial inactivation of the weakest test parasites, the water sample controls or dry controls would have been a confirmation that the rapid decrease in parasite recovery was not due to the inability to separate the test parasites from the solids after 3 months storage. The validity of the study, however, is not compromised in terms of the inactivation results on a comparative basis and the 25°C storage condition, in conjunction with storage time, virtually inactivated the digester resistant parasites in sewage sludge.

In summary, the study protocol consisted of the following:

(1) The eggs of three digester resistant nematodes (Ascaris suum, Toxocara canis, and Trichuris spp., and a cestode, Hymenolepis diminuta) were added after anaerobic sludge digestion. (These digested sludge samples are designated--anaerobic sludge samples--Phase I).

(2) Similarly the eggs of the same 3 nematodes and the cestode were added to digested sludges after aerobic digestion. (These sludge samples are designated--aerobic sludge samples--Phase I).

(3) The eggs of the same 3 nematodes and the cestode were added to the initial raw sludge and during anaerobic digestion of the sludge. (These sludge samples are designated--anaerobic sludge samples--Phase II).

(4) The eggs of the same 3 nematodes and the cestode were added to the initial raw sludge and during aerobic digestion of the sludge. (These sludge samples are designated--aerobic sludge samples--Phase II).

Phase II sludges are the most analogous to a real situation in which parasite eggs are introduced to sludges during digestion processes. The results of Phase I sludges in which eggs were added after sludges had been digested and before storage occurred were used to test whether or not the digestion processes of Phase II sludges had any effect on the recovery, viability and infectivity of the parasite eggs tested (that is, the eggs in Phase I sludges were not subjected to the digestion processes). Soil controls were used to test whether or not the composition of the sludges themselves had any effect on the inactivation, viability and infectivity of the eggs. It was noted that although eggs often appeared viable, they did not necessarily produce infections in test rats.

The Phase I and Phase II digested sludge and pseudo-soil samples were stored under the following conditions:

(a) at a constant temperature of 25°C
(b) at a constant temperature of 4°C
(c) and in drums that were inserted in the ground

Samples of the digested sludges and pseudo-soil controls were analyzed at the beginning of the storage period and every 3 months thereafter up to about 24 months and after 30 and 33 months storage for a select number of test samples. Samples were analyzed for parasite recovery, parasite viability, and infectivity.

Each of the above sequence of the test protocol is discussed in more detail in the following sub-paragraphs of this section.

TEST PARASITES

Toxocara canis and Trichuris vulpis eggs were recovered from the feces of naturally infected dogs obtained from the Society for the Prevention of Cruelty to Animals (Cincinnati, OH). The dogs were housed in the Laboratory of Animal Medicine at the University of Cincinnati. Additional feces from naturally infected dogs were obtained from the Findlay Humane Society (Findlay, OH). Ascaris suum and Trichuris suis were recovered from the intestinal contents of naturally infected pigs obtained from the Pioneer Packing Plant (Bowling Green, OH) and the Dinner Bell Packing Plant (Archibold, OH). Hymenolepis diminuta eggs were recovered from the feces of rats infected by feeding them adult Tenebrio beetles containing cysticercoids (Carolina Biological Supply Co., Burlington, NC).

Isolation of the eggs from the fecal material was according to the following protocol. Approximately 100 g of feces were soaked in 1 liter of distilled water for 11 to 12 hours (13). The material was then washed through a U.S.A. Standard Testing Sieve No. 10. The filtrate containing the eggs was allowed to settle for 1 hour. The supernatant was decanted and discarded. The sediment was then passed through a No. 18 sieve.

Settling, decanting and sieving were repeated until the sediment had been filtered through No. 25, 35, 60, 100 and 150 sieves. Following this sieving step, the sediment was suspended in 1 liter of distilled water; allowed to settle for 1 hour and the supernatant decanted and discarded. This washing procedure was repeated four times. Following the final wash, 30 ml of sediment was placed in 250 ml plastic centrifuge bottles. The bottles were filled with a 71% sucrose solution, (sp. gr. 1.26) capped and mixed. The mixture was centrifuged at 100 X g for 5 minutes at 4°C in a swinging bucket rotor (IEC PR-2 preparative centrifuge; I.E.C., Needham Heights, MA). The eggs at the top of the liquid were removed and washed four times with distilled water in conical tubes by centrifugation at 550 X g for two minutes. The isolated eggs were then stored at 4°C until sufficient numbers were accumulated.

In total, 2.1×10^7 *Ascaris suum*; 2.6×10^7 *Hymenolepis diminuta*; 4.3×10^6 *Toxocara canis* and 2×10^6 *Trichuris* spp. eggs were collected. Each egg total was divided into seven equal aliquots: 1) eggs to be added to aerobically digested sludge (Phase I aerobically digested sludge samples); 2) eggs to be added to anaerobically digested sludge (Phase I anaerobically digested sludge samples); 3) eggs to be added prior to aerobic digestion (part of Phase II aerobically digested sludge samples); 4) eggs to be added prior to anaerobic digestion (part of Phase II anaerobically digested sludge samples); 5) eggs for daily aerobic feed (also part of Phase II aerobically digested sludge samples); 6) eggs for daily anaerobic feed (also part of Phase II anaerobically digested sludge samples); and 7) eggs to be added to the soil (control) samples (Table 1).

TABLE 1. NUMBER OF EGGS ADDED TO SLUDGES

Number of Eggs Added Per 30 Liters of Sludge After Digestion (Phase I), Prior to Phase II Digestion, and to Soil Samples

Ascaris	*Toxocara*	*Hymenolepis*	*Trichuris*
3.1×10^6	6.2×10^5	3.7×10^6	2.8×10^5

Number of Eggs Added Each Day During the 15 Day Digester (Daily Draw and Fill) Retention Period

Ascaris	*Toxocara*	*Hymenolepis*	*Trichuris*
2.0×10^5	4.1×10^4	2.5×10^5	1.8×10^4

ADDITION OF TEST PARASITES TO DIGESTED SEWAGE SLUDGE AND SOIL CONTROLS

The procedure employed to add the test parasites to the laboratory sewage sludge digesters (either anaerobic or aerobic) is documented in reference 14. This study also reported on the survival of the test parasites after either aerobic or anaerobic sludge digestion. The test parasites were added two different ways to the sludges; the first, after either aerobic or anaerobic sludge digestion was completed; the second, prior to and by the daily feeding of raw sludge during aerobic or anaerobic sludge digestion. In the latter procedure, the actual parasite-seeded digested sludge samples used for the storage tests were the digested sludges in the digester units on the last day of the treatment. A summary of the operation of the sludge digesters, the addition of eggs to the digesters and soil control is presented in the following paragraphs.

Sludge Digesters

In December, 1979, four 15-liter capacity aerobic digesters were started in the Environmental Engineering Department of the University of Cincinnati with activated sludge from the Muddy Creek Treatment Plant, Cincinnati, Ohio. The Muddy Creek Plant treats primarily domestic wastes and the sludge generated is aerobically digested. Each of the laboratory aerobic digesters had two diffusers that delivered a minimum air flow of 0.4 l per minute (0.015 cubic feet per minute). These laboratory aerobic digesters were fed primary and secondary sludges from the Muddy Creek Plant. Sludge feed for the aerobic digesters was wasted secondary sludge which was thickened by adding gravity thickened sludge (mostly primary) to achieve a level close to 3% total solids.

Four 15-liter capacity anaerobic digesters were started with 4 liters of anaerobic sludge and 11 liters of primary sludge from the Sycamore Treatment Plant near Cincinnati, Ohio. This plant treats domestic wastewater and the sludge generated is anaerobically digested. After startup, the daily sludge feed to these digesters was primary sludge from the Muddy Creek Treatment Plant, Cincinnati, Ohio. Thus the same sludge source was used for the feed to both anaerobic and aerobic digesters. These laboratory anaerobic digesters were operated under mesophilic conditions in a 35^{o}C constant temperature room. In the first 6 weeks of operation, the sludge feed was steadily increased to adjust the sludge retention time to 15 days. The next 30 days were a period of operation under a constant feed rate to allow the anaerobic digesters to come to equilibrium before the parasites spiking operations began. The anaerobic digesters were sealed gas-tight and mixed 5 minutes per hour by pumping the enclosed gases through the sludge. This procedure achieved thorough sludge mixing. The digesters were operated on a daily draw and fill basis. The anaerobic digester feed was primary sludge, diluted when necessary or thickened with gravity thickened primary sludge to achieve a level of nearly 5% total solids.

Two of each type of digesters were needed to achieve the 30 liters of sludge required for the storage tests. The extra digesters were operated

as backups. Select parameters were monitored during the operation of the digesters. Except for the volatile acids, all tests were conducted according to Standard Methods, 14th Edition, American Public Health Association.

The alkalinity of the supernatant was measured using methyl purple for the end point. It was believed that this method was as precise as a potentiometric determination on whole sludge. The alkalinity was difficult to measure because the sludge solids interfered with electrode response.

The amount of solids in both aerobically and anaerobically digested sludges was determined by placing an aliquot of sludge into a weighed dish and drying to a constant weight at 103 to 105°C. The increase in weight, over that of the empty evaporating dish, represents total solids in the aliquot. Aerobically digested sludge contained 2.0 to 2.9% (mean = 2.45%), total solids and anaerobically digested sludge contained 2.9 to 3.4% (mean = 3.15%) total solids.

The anaerobic digesters achieved a volatile suspended solids reduction of 23.8% and a Chemical Oxygen Demand (COD) reduction of 54.9%. The pH was stable and volatile acids levels stabilized at a low level, indicating good digestion activity. The aerobic digesters achieved a volatile suspended solids reduction of 16.4% and a COD reduction of 37%. These reductions could have been higher if the aerobic digesters were operated on a batch instead of a semi-continuous basis.

Addition of Eggs to the Digesters Before and During Aerobic and Anaerobic Sludge Digestion (Phase II Digested Sludges)

The following discusses the procedure used to add the test parasites prior to and during the daily draw and feed of the sludge to the ongoing anaerobic or aerobic digesters.

After the anaerobic digesters were stabilized, a daily wasting and feeding of sludge and parasite eggs were made for 15 consecutive days. This operation was accomplished by means of two valves, one on top and one on the bottom of each digester. First, the lower valve was opened and 1 liter of wasted sludge was collected. Then this lower valve was closed. The aliquot of eggs and the day's feed sludge was poured into a funnel above the upper valve. This valve was opened only after the feed had filled the funnel, thus minimizing the amount of air introduced into the anaerobic system.

The feeding and wasting of the aerobic digester were performed in the same order, except no precautions were taken to prevent air from entering the feed. The digesters were mixed during the feeding and wasting operations and for 30 to 40 minutes to ensure the homogeneous distribution of the eggs.

Addition of Parasite Eggs After Aerobic and Anaerobic Sludge Digestion (Phase I Digested Sludges)

Following a 15 day retention time, all aerobic digester contents (30 liters) were pooled and all anaerobic digester contents (30 liters) were pooled. The test parasite eggs were added to each type of digested sludge. The sludge was mixed with a 8-mm diameter multi-hairpin-looped metal rod for 1 hour.

Effectiveness of Sludge Digestion for Inactivating Parasites

The digester seeding study was also used to estimate the amount of parasite inactivation resulting from the laboratory controlled aerobic and anaerobic sludge digestion (14). Approximately 23% of the Ascaris eggs were inactivated during anaerobic sludge digestion and 38% during aerobic sludge digestion. About 11% of the Trichuris eggs were inactivated during aerobic digestion. Trichuris eggs apparently survived anaerobic sludge digestion. Toxocara eggs apparently survived well during either aerobic or anaerobic sludge digestion, suggesting that this parasite could be more resistant to sludge digestion than Ascaris eggs. It also appeared that parasite inactivation was more effective during aerobic digestion as compared to anaerobic digestion. The inactivation of some of the parasite eggs during the sludge digestion was also helped by the embryonation of some of the Ascaris and Trichuris eggs in the intestinal system or feces of the host, and therefore were past their most resistant stage. Also, eggs of Hymenolepis diminuta apparently became non-viable before addition to the digesters and therefore their usefulness for this study was decreased.

Parasite-Seeded Soil Control

A non-sludge control was included as part of this parasite inactivation study. The test parasites were added to the soil controls and stored under the same conditions along with the parasite-seeded digested sludges. These non-sludge controls were analyzed for parasite eggs (total and viable) at the same time as the parasite-seeded sludge samples. Top soil was used for the non-sludge control. A mixture of 1 part loam; 1 part manure; and 1 part clay was used in an effort to duplicate naturally occurring "Top Soil" in Cincinnati, OH. It had been determined that the aerobically digested sludges contained 2.0 to 2.9 g solids per 100 g wet weight and the anaerobically digested sludges contained 2.9 to 3.4 g solids per 100g wet weight. Therefore, the soil controls were made up to contain about 3% top soil solids in water.

Three hundred grams each of loam, manure and clay were added to 29.1 liters of distilled water and mixed for 1 hour. The following approximate amounts of parasite eggs were added to this mixture: 1) 3.0×10^6 Ascaris suum eggs; 2) 3.7×10^6 Hymenolepis diminuta eggs; 3) 6.0×10^5 Toxocara canis eggs; and 4) 2.9×10^5 Trichuris spp. eggs. These additions were about the same numbers of parasite eggs that were added to 30 liters of each type of digested sludge. The top soil, parasite eggs and water were mixed for 1 hour.

PREPARATION OF THE PARASITE-SEEDED DIGESTED SLUDGE SAMPLES FOR THE STORAGE TESTS

One hundred grams (wet weight) of parasite-seeded digested sludge samples were placed into pre-sterilized, 250 ml polypropylene bottles and sealed with screw caps (Nalge Company, Rochester, NY). The sludge was continuously mixed during this aliquoting step. The initial number of parasite eggs in the digested sludge samples are summarized in Table 2. Each batch of sludge and soil samples was divided into 288, 100-g (wet weight) aliquots. The caps were left slightly unscrewed to allow air movement to and from the stored test sample bottles.

The number of digester sludge test conditions was 12; that is, two parasite-seeded aerobically digested sludge sample groups--Phase I and Phase II--and two anaerobically parasite-seeded digested sludge sample groups--Phase I and Phase II; each stored under three test conditions--at a constant temperature of 25°C and 4°C, and in drums that were inserted in the ground.

A sufficient number of parasite-spiked digested sludge samples was thus available for the beginning of the storage tests (time 0) and every three months thereafter for at least 33 months (11 sampling periods after time 0). Eight of the parasite-digested sludge samples were available for analyses for each of the 12 test conditions. The parasite analyses consisted of determining the parasite recovery, viability, and the infectivity of Ascaris and Toxocara eggs recovered from the test samples. Thus, a total of 96 test parasite-seeded digested sludge samples were analyzed at each sampling period. This number of parasite-spiked digested sludge samples was designed to allow for statistical interpretation of the parasite inactivation effects and preparation of plots showing inactivation effects with time.

In a similar fashion, 100-g (wet weight) parasite-spiked soil control aliquots were placed into pre-sterilized, 250 ml polypropylene bottles and sealed with screw caps. The top soil, parasite eggs and water were continuously mixed during the aliquoting step. The initial number of parasite eggs in the soil control samples are also shown in Table 2. These parasite-spiked soil controls were stored along with the parasite-spiked digested sludge samples under each of the three storage conditions. Eight of the parasite-spiked soil controls were taken for the same analyses as the parasite-spiked digested sludges for each of the storage conditions at the same time intervals (every 3 months for 33 months). Thus, a total of 24 parasite-spiked soil controls were sampled along with the total of 96 parasite-spiked digested sludge samples at each time period.

The 25°C samples were stored in a temperature monitored controlled room and the temperature recorded (Taylor Instrument Company, Rochester, NY). The 4°C samples were stored inside a Thesco Schimdt Constant Temperature Cold Room. The samples placed in 55 gallon galvanized drums that were buried in the ground were to simulate a sludge storage lagoon subjected to the temperature of Cincinnati, OH environment. The air and drum temperatures were recorded with a Weathertronics 4120 Thermograph (Weathertronics, Sacramento, California).

TABLE 2. NUMBER OF PARASITE EGGS PRESENT IN SAMPLES AT TIME 0 (EITHER ADDED TO DIGESTED SLUDGES OR MEASURED AFTER DIGESTION WAS COMPLETED)

A. Number of Eggs in Aerobically Digested Sludge: Phase I

	Eggs/100g Wet Weight	Eggs/g Dry Weight
Ascaris	10,353	4,226
Hymenolepis	12,503	5,103
Toxocara	2,073	846
Trichuris	931	380

B. Number of Eggs in Anaerobically Digested Sludge: Phase I

	Eggs/100g Wet Weight	Eggs/g Dry Weight
Ascaris	10,353	3,287
Hymenolepis	12,503	3,969
Toxocara	2,073	658
Trichuris	931	296

C. Number of Eggs in Aerobically Digested Sludge: Phase II

	Eggs/100g Wet Weight	Eggs/g Dry Weight
Ascaris	4800 $\pm$ 176*	2000 $\pm$ 72*
Hymenolepis	4800 $\pm$ 199	2000 $\pm$ 81
Toxocara	1700 $\pm$ 127	700 $\pm$ 52
Trichuris	400 $\pm$ 40	100 $\pm$ 16

(continued)

TABLE 2. (Continued)

D. Number of Eggs in Anaerobically Digested Sludge: Phase II

	Eggs/100g Wet Weight	Eggs/g Dry Weight
Ascaris	5300 ± 475*	1685 ± 151*
Hymenolepis	7500 ± 535	2380 ± 170
Toxocara	1500 ± 168	480 ± 53
Trichuris	900 ± 63	285 ± 20

E. Number of Eggs in Soil Samples

	Eggs/100g Wet Weight	Eggs/g Dry Weight
Ascaris	10,353	3,451
Hymenolepis	12,503	4,167
Toxocara	2,073	691
Trichuris	931	310

*Standard Error N=3

RECOVERY OF PARASITE EGGS FROM SLUDGE AND SOIL SAMPLES

A sludge sample (100 g wet weight) was filtered through a U.S.A. Standard Testing Sieve No. 35 and washed with distilled water. The filtrate was returned to its bottle and centrifuged at 400 X g for 5 minutes at 4°C. The supernatant was decanted, and 100 ml of 3% (w/v) lactalbumin hydrolysate was added to inhibit adherence of sludge material to eggs in the centrifuge pellet. This mixture was washed free of lactalbumin hydrolysate with distilled water and then resuspended in distilled water to a final volume of 50 ml. This suspension was layered onto a 150 ml continuous sucrose density gradient (sp. gr. 1.26 to 1.00) and centrifuged at 800 X g for 15 min. After the pellet was discarded, the material in the gradient was collected, diluted with an equal volume of distilled water and centrifuged at 800 X g for 5 min. All the pelletable material from a single 100-g sample was pooled and washed 3 times with distilled water.

To determine the efficiency of this method for recovering eggs from sludge 6 samples of aerobically digested sludge were autoclaved to destroy any eggs potentially present in the sludge, then these samples were inoculated with 10 Ascaris lumbricoides eggs that had been removed from the uteri of adult females (Carolina Biological Supply Company, Burlington, NC). Processing of three of these samples gave a recovery of 78 $\pm$ 15 percent. Recovery tests for anaerobic sludges and soil controls were not done but were assumed to be similar.

METHOD FOR DETERMINING VIABILITY OF PARASITE EGGS

To properly assess the inactivation of parasite eggs during storage, it was necessary to incubate the recovered eggs. Parasite ova may fail to embryonate when held in sludge. Eggs recovered from sludge may appear to be dead but may be healthy and viable when given time and the proper environment in which to embryonate (15). Thus, the washed pellet containing the eggs (see prior section) was suspended in 5 ml of 0.3 N sulfuric acid to retard the growth of molds, bacteria and other microorganisms. These samples were placed into a plastic Petri dish (100 X 15 mm). The Petri dishes were placed in a covered container humidified by including a small beaker of water in it. The eggs were incubated at 25°C in the dark for 30 days, the length of time necessary for the parasite eggs to reach the potentially infective larval stage.

After 30 days, the samples were examined for the total number of parasite eggs recovered and for those eggs that embryonated, using a Sedgewick-Rafter Counting Chamber and a Whipple ocular disk. An egg was considered embryonated if a larva was visible within the egg shell or if earlier developmental stages were recognized. The morphological changes appearing in the parasite eggs that were used as criteria of dead eggs were: 1) cytolysis of egg cell (ovum) or cells (after cleavage had started); 2) vacuolation in the cytoplasm of the egg; 3) formation of large refractile granules within the cell; 4) cytoplasmic hyalinization; 5) shrinkage of the egg; 6) disintegration of the membrane surrounding the egg cell (ovum); and 7) caving in of the egg shell.

METHOD FOR DETERMINING INFECTIVITY OF PARASITE EGGS

Samples containing the viable and non-viable eggs were concentrated to 2 ml and intubated into male Holtzman albino rats (Charles River Breeding Laboatories, Inc., Wilmington, MA). Too few Trichuris eggs were recovered to enable their use in infectivity studies. After 8 days, the exposed rats were sacrificed with ether and their lungs, liver, kidneys and brain removed. Each organ was placed into 50 to 100 ml of physiological saline solution (0.85% sodium chloride) and cut into pieces with surgical scissors no bigger than 3 to 5 mm in the longest dimension. One Baermann apparatus was set up for each organ (16). The funnel was filled with saline (37°C). A two layer piece of cheesecloth was used to suspend the minced organ within the saline solution of each funnel. Lamps with 60 watt tungsten bulbs were placed approximately 5.08 cm (2 in.) from the junction of the head and stem of each funnel. After 50 to 60 min, the suspended organ was removed and discarded. The contents of each funnel were placed into 50 ml

glass conical graduated centrifuge tubes. Ten ml of 0.2% saponin was added to each tube to lyse red blood cells (17). The tubes were centrifuged for 2 min at 500 X g and the supernatant was discarded. The volume of pellet remaining in each tube was examined. A small pellet (less than 0.5 ml) required no further treatment and was resuspended in 20 ml of a 0.1% Tween-80, 10% formalin-saline solution (5.4 ml 37% formalin in 14.6 ml 0.1% (w/v) Tween-80 in physiological saline solution) to fix and preserve the larvae. Fixed samples were stored at 4°C until examined for larval content.

Tubes containing 0.5 ml or more of tissue pellet required further reduction before they could be analyzed. These pellets were suspended in 30 ml of pepsin digest solution [2.5 g pepsin (Sigma P-7000; 1:10,000) in 0.85% (w/v) sodium chloride plus 3.5 ml concentrated hydrochloric acid] and incubated at 30°C for 24 hours on a platform rotating at 200 RPM (18). The digested tissue was rinsed free of the pepsin solution with distilled water and fixed and stored as above. Each sample was examined under 125 X magnification with a phase contrast microscope and the number of Ascaris and Toxocara larvae noted.

SECTION 5

ADDITIONAL STUDIES

SUPPLEMENTAL STUDY 1: STAINING WITH DYES FOR TESTING PARASITE VIABILITY

Initially we also proposed to test parasite viability by vital dye staining and/or dye exclusion. Numerous vital dyes (dyes that stain only living cells) as well as dyes that are excluded from living cells but are incorporated into dead ones were tested as indicators of viability. The dyes tested were: Trypan Blue, Nile Blue Sulfate, Methylene Blue, Methyl Violet 2 B, Crystal Violet, Janus Green B, Brilliant Red, Benzopurpurin, Saphranin O, Carmin Alum Lake, Brilliant Cresyl Blue and Neutral Red. Also, commercially available Nile Blue Sulfate contains two components which were separated out in various solvents and tested for their ability to detect viable parasite eggs. Various fractions were collected as eluates off silicic acid columns. The acetone fraction (containing mainly Nile Pink) and the chloroform fraction (containing mainly Nile Blue) were tested. None of these dyes proved satisfactory because no single dye was able to differentiate between viable and non-viable eggs of all of the three nematodes and the cestode.

SUPPLEMENTAL STUDY 2. MEASUREMENT OF ORP (OXIDATION-REDUCTION POTENTIAL)

Oxidation-Reduction Potential (ORP) measurements were made on a limited number of parasite-spiked sludge and soil control samples in the hope of relating the changes in parasite levels to ORP.

The method employed for the determination of the ORP of the sludge and soil was obtained from: ASTM Designation: D-1498-76; Standard Practice for Oxidation- Reduction Potential of Water.

A Fisher Research Acumat pH meter, equipped with a Hg, Hg_2Cl_2, saturated KCl reference electrode and a platinum oxidation-reduction electrode, was used for the ORP measurements. Before use, the meter was allowed to warm up thoroughly (20 to 30 min). Also, before measuring the ORP of each sample, the response of the electrodes to the standard ferrous-ferric reference solution was checked. The electrodes were washed with a stream of double distilled water from a wash bottle. The electrodes were then immersed in a clean beaker containing fresh standard ferrous-ferric reference solution. The asymmetry control was adjusted to the millivolt potential of the ferrous-ferric reference solution (+430 mV). Without changing the asymmetry potential control, the washing and reading procedures were repeated until two successive readings were constant.

After the system was standardized, the sample bottle was swirled quickly to mix the supernate and sediment; and the sample contents poured into a clean glass beaker. Subsequently, the electrodes were inserted into the sample and the ORP read directly from the meter scale and recorded. Eight samples from each test condition were measured.

SECTION 6

RESULTS

The data were analyzed to determine whether each of the various storage conditions has an influence on the recoverability and viability of the test parasite eggs. Two-factorial analysis of variance (ANOVA) with repeated measures was employed (BMDP Statistical Software, Inc., University of California). The first ANOVA (Table 3) used an abridged set of data. The data eliminated from these analyses were those of months 0, 19, 30 and 33 because complete data for the parasite-spiked sludge samples were not available. Also, in this ANOVA data for the parasite-spiked soil samples were not included because these data may not be comparable to sludge samples. The soil samples were not treated aerobically or anaerobically and eggs were not added to the soils over a period of 15 consecutive days (as would be required for a strict statistical analysis of variance evaluation).

Nevertheless, in the real world soils are not normally digested aerobically or anaerobically to stabilize against putrification or to reduce vector attraction. Also, the test parasites were added directly to the soil samples similar to the test parasites added to the sludges after digestion and exposed to the same temperatures and storage time conditions. It was felt, therefore, that useful information would be available by performing an ANOVA (Table 4) that included the test parasite soil samples and thereby enable comparisons with the ANOVA (Table 3) that excluded the soil samples. Again, data for months 0, 19, 30 and 33 were not included because complete data sets were not available.

The data used in the ANOVA's and in Figures 5 through 21 were the actual number of test parasite eggs recovered and the number of viable eggs observed per 100 g wet weight of sample. The geometric means and standard deviations (in geometric terms, multiplication and division) are shown in the appendix Tables A-1 through A-21. The tic marks in the figures represent the geometric standard deviations for the recovery of the test parasites. Geometric standard deviations were not shown for the viable eggs data in the interest of not cluttering the figures. Also, tic marks were not shown for the Hymenolepis sp. egg recovery because only recovery information was available. The Hymenolepis eggs viability were compromised during initial storage before their addition to the digested sludges and soils.

Included in Tables A-1 through A-21 are the available geometric means and standard deviations for the test parasite eggs recovered and viable for

TABLE 3: FACTORIAL ANALYSIS OF VARIANCE
(Soil Data not Included)

Test Parasite	Source of Variability *			
	Aerobic versus Anaerobic Digested Sludge	Phase I versus Phase II Digested Sludge	Sludge Temperature	Storage Time
Ascaris (recovered)				
F	0.00	7.53	8.14	8.68
Tail Probability	0.9648	0.0062	0.0003	0.00
Ascaris (viable)				
F	45.25	0.65	51.49	32.33
Tail Probability	0.00	0.42	0.00	0.00
Toxocara (recovered)				
F	0.67	0.11	9.33	4.63
Tail Probability	0.4124	0.7421	0.0001	0.0001
Toxocara (viable)				
F	20.25	1.41	389.57	66.87
Tail Probability	0.00	0.2348	0.00	0.00
Trichuris (recovered)				
F	27.56	42.69	7.46	9.55
Tail Probability	0.00	0.00	0.0006	0.00
Trichuris (viable)				
F	0.75	2.45	8.18	26.83
Tail Probability	0.3857	0.1179	0.0003	0.00
Hymenolepis (recovered)				
F	9.14	1.47	65.13	11.05
Tail Probability	0.0026	0.2265	0.00	0.00

* Degrees of Freedom: Sludge Type = 1, Phase I versus Phase II = 1, Storage Temperature = 2, Storage Time = 6

TABLE 4: FACTORIAL ANALYSIS OF VARIANCE
(Soil Data Included)

Test Parasite	Source of Variability *			
	Aerobic/Anaerobic Digested Sludge Treatment	Phase I/Phase II with Soil Treatment	Sludge Temperature	Storage Time
Ascaris (recovered)				
F	4.52	84.66	11.75	6.87
Tail Probability	0.0338	0.00	0.00	0.00
Ascaris (viable)				
F	37.15	37.32	98.83	37.69
Tail Probability	0.00	0.00	0.00	0.00
Toxocara (recovered)				
F	0.37	29.36	24.68	4.61
Tail Probability	0.5443	0.00	0.00	0.0001
Toxocara (viable)				
F	43.48	85.63	333.39	64.19
Tail Probability	0.00	0.00	0.00	0.00
Trichuris (recovered)				
F	39.83	31.10	9.80	9.68
Tail Probability	0.00	0.00	0.0001	0.00
Trichuris (viable)				
F	0.11	12.13	13.91	21.47
Tail Probability	0.7412	0.00	0.00	0.00
Hymenolepis (recovered)				
F	0.01	160.15	164.12	19.05
Tail Probability	0.9328	0.00	0.00	0.00

* Degrees of Freedom: Sludge Type = 1, Phase I vs. Phase II vs Soil = 2, Storage Temperature = 2, Storage Time = 6

times 0 (estimated for the beginning of the storage period), 19, 30 and 33 months. Figures 5 through 21 show the recovery and viability for the same sampling times as used in the ANOVA's, that is after 3, 8, 10, 13, 16, 22 and 25 months of storage. If the reader desires, time 0 test parasite eggs levels could be taken as the amount of eggs initially added (Table 2) to the sludges and soil samples. The slope between time 0 and 3 months storage will change in the figure, but not the ANOVA's because time 0 was excluded. The reader also may wish to consider the data in terms of percent recovered and percent viable for the parasite eggs. Percent recovered can be calculated from the information in appendix Tables A-1 through A-21 starting at the beginning of the storage tests or by using the parasite levels in Table 2 if the initial levels of the test parasites' eggs added to the sludges are desired. Percent viability of eggs at each time of sampling also can be calculated using the appendix Tables A-1 through A-21 or Figures 5 through 21.

As indicated previously the test parasite samples were stored under three different conditions: 25°C, 4°C and in drums stored in the ground. The purpose of the test samples placed in the 55-gallon galvanized drums buried in the ground were to simulate a sludge storage lagoon subjected to the temperate climatic conditions of Cincinnati, Ohio. The air and drum temperatures were recorded (Figure 1) with a Weathertronics 4120 thermograph (Weathertronics, Sacramento, California).

A summary of the viability and infectivity of recovered Ascaris and Toxocara eggs is shown in Tables 5 and 6. The data on infectivity are qualitative and not quantitative because only negative versus positive infections of the test rats were determined. Also, the test rats are not the normal host for the Ascaris and Toxocara eggs, and were used only as an indication of infectivity. A negative (-) indicates that no larval forms of the parasite were found in the tissues examined. In some instances, hemorrhaging was noted in the lungs of the sacrificed rat; this alone was not considered evidence of parasite infection. The presence of larval forms was necessary. It was noted that although eggs often appeared viable, they did not necessarily produce infections in test rats.

Figures 2, 3, and 4 show the changes in pH of the test parasite soil and sludge samples with storage time. The effect of pH will be discussed in the next section (Discussion of Results). Also the staining with dyes and the oxidation-reduction potential measurement studies will be discussed in the next section.

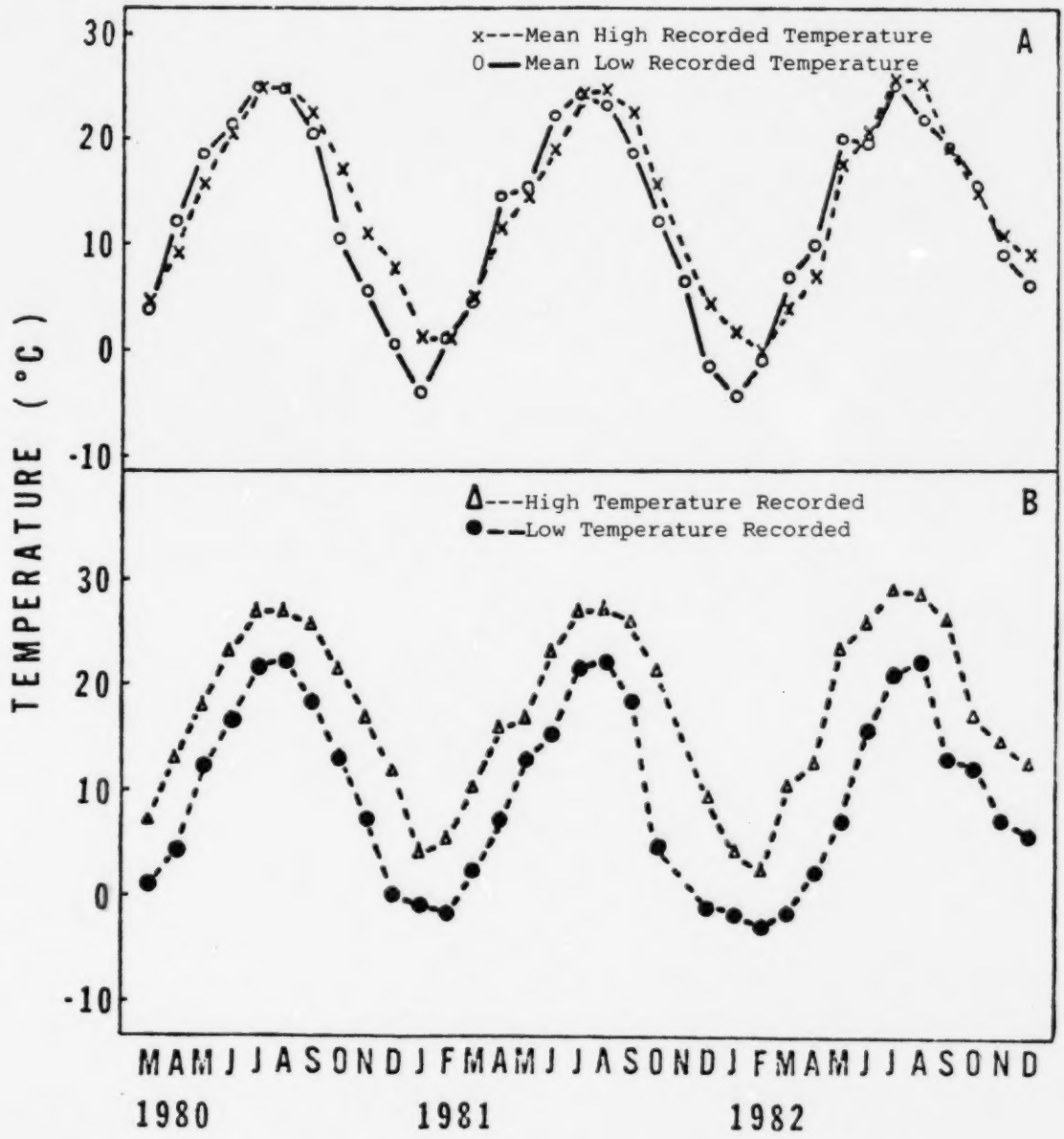

Figure 1. Monthly Variation of Air and In-ground Storage Temperature

A. Air Temperature

B. In-ground Drum Storage Temperature

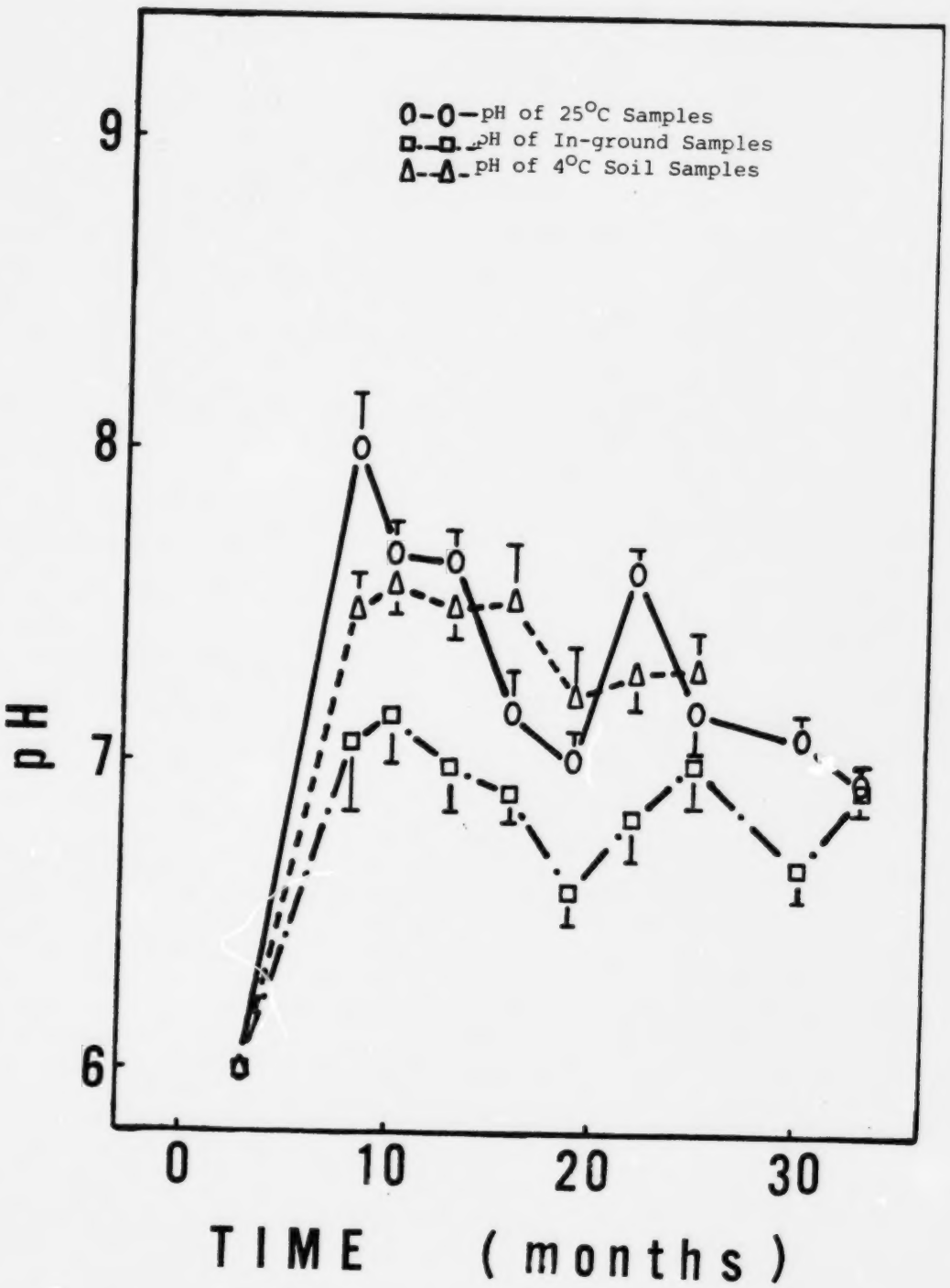

Figure 2. Changes in pH of Soil Samples with Storage Time

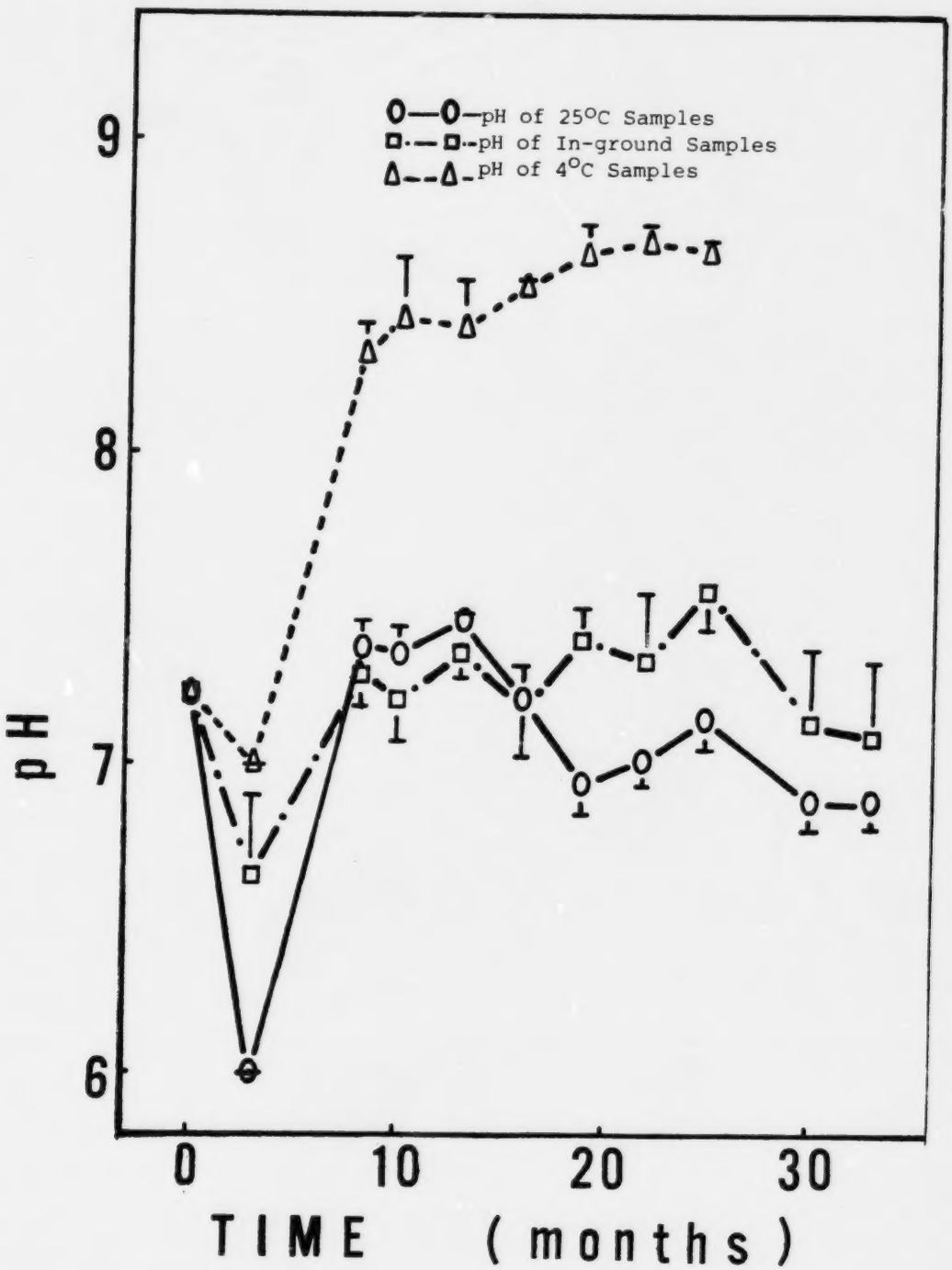

Figure 3. Changes in pH of Aerobic Digested Sludge with Storage Time

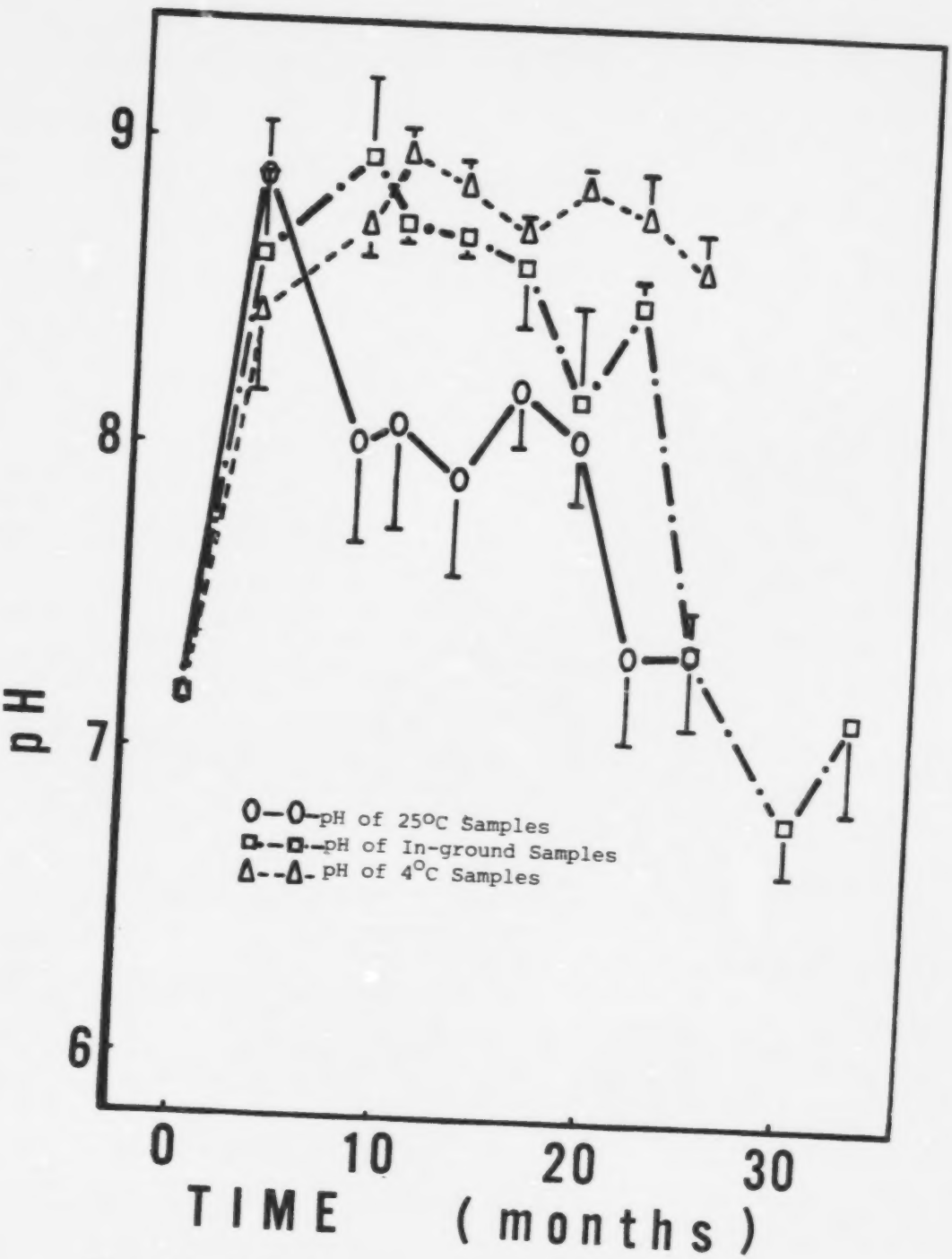

Figure 4. Changes in pH of Anaerobic Digested Sludge with Storage Time

SECTION 7

DISCUSSION OF RESULTS

GENERAL

Upon examining Figures 5 through 21 and Tables A-1 through A-21, it is evident that the recoveries of parasite species were much lower after 3 months of storage. This apparent decrease in the recovery of parasites eggs may be due to: (1) an actual loss or destruction of parasite eggs, (2) a defect (or defects) in the parasite egg recovery method or (3) a lack in homogeneity in the original suspensions; and although the suspensions were thoroughly mixed, it is possible that the dispersions of parasite eggs were not complete. Also, not all of the Time 0 (initial storage) samples were directly measured; the values in the tables are estimates (see page 63 APPENDICES).

ANALYSIS OF VARIANCE

The overwhelming effects that stand out in the ANOVA's of Tables 3 and 4 are that storage temperature and storage time are highly significant in the recovery and viability of the test parasite eggs. Further examination of Figures 5 through 21 and appendix Tables A-1 through A-21 show that storing sludges and soils at 25°C resulted in lower recovery and viability of the test parasites as compared to storage in the ground and at 4°C. The one exception is the lack of inactivation of Toxocara eggs in the soil samples at 25°C (Figure 14). Why the lack in the reduction of the viability of Toxocara eggs in this environment is uncertain.

Review of Figures 5 through 21 and appendix Tables A-1 through A-21 suggest that the test parasites in the ground stored samples also will be substantially inactivated in the digested sludges given sufficient time under the Cincinnati climatic environment. AT least about 33 months of storage is needed for inactivation of the test parasites in the digested sludges stored under the Cincinnati temperate climatic environment.

The ANOVA shown in Table 3, which excludes the soil data, indicate a significant difference in the viability of Ascaris and Toxocara eggs in aerobic versus anaerobic digested sludges. There was no significant difference, however, in the recovery of these eggs in either aerobic or anaerobic digested sludges. The recovery and viability of these eggs are shown in Figures 5 through 8 and 10 through 13. It would appear that the significant effect occurs in the viability of these eggs in the digested sludges stored in-ground. The decrease in the viability of Ascaris and Toxocara eggs is much slower in aerobic as compared to anaerobic digested

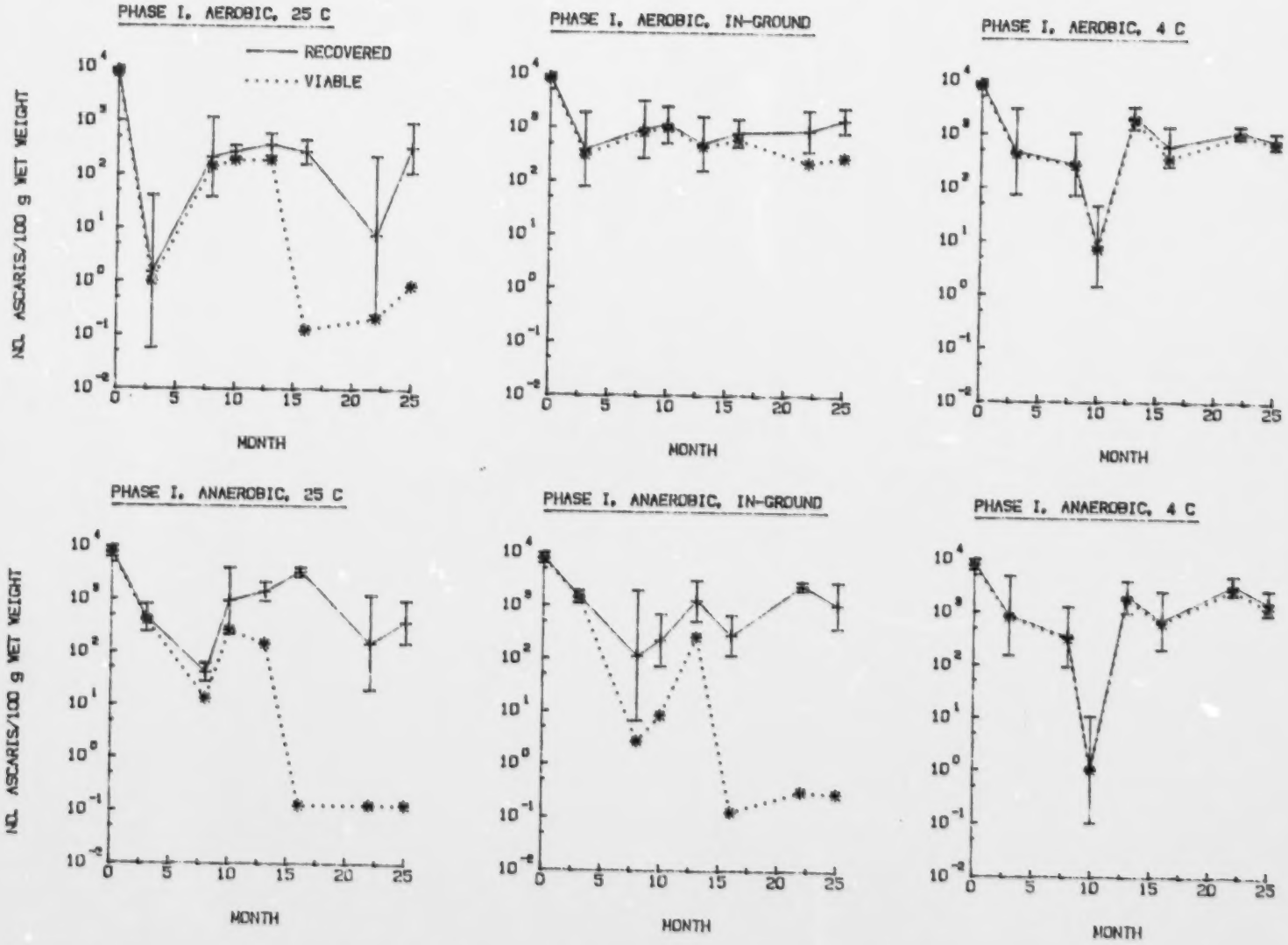

Figure 5. Recovered and viable Ascaris eggs in Phase I aerobic and anaerobic sludge samples.

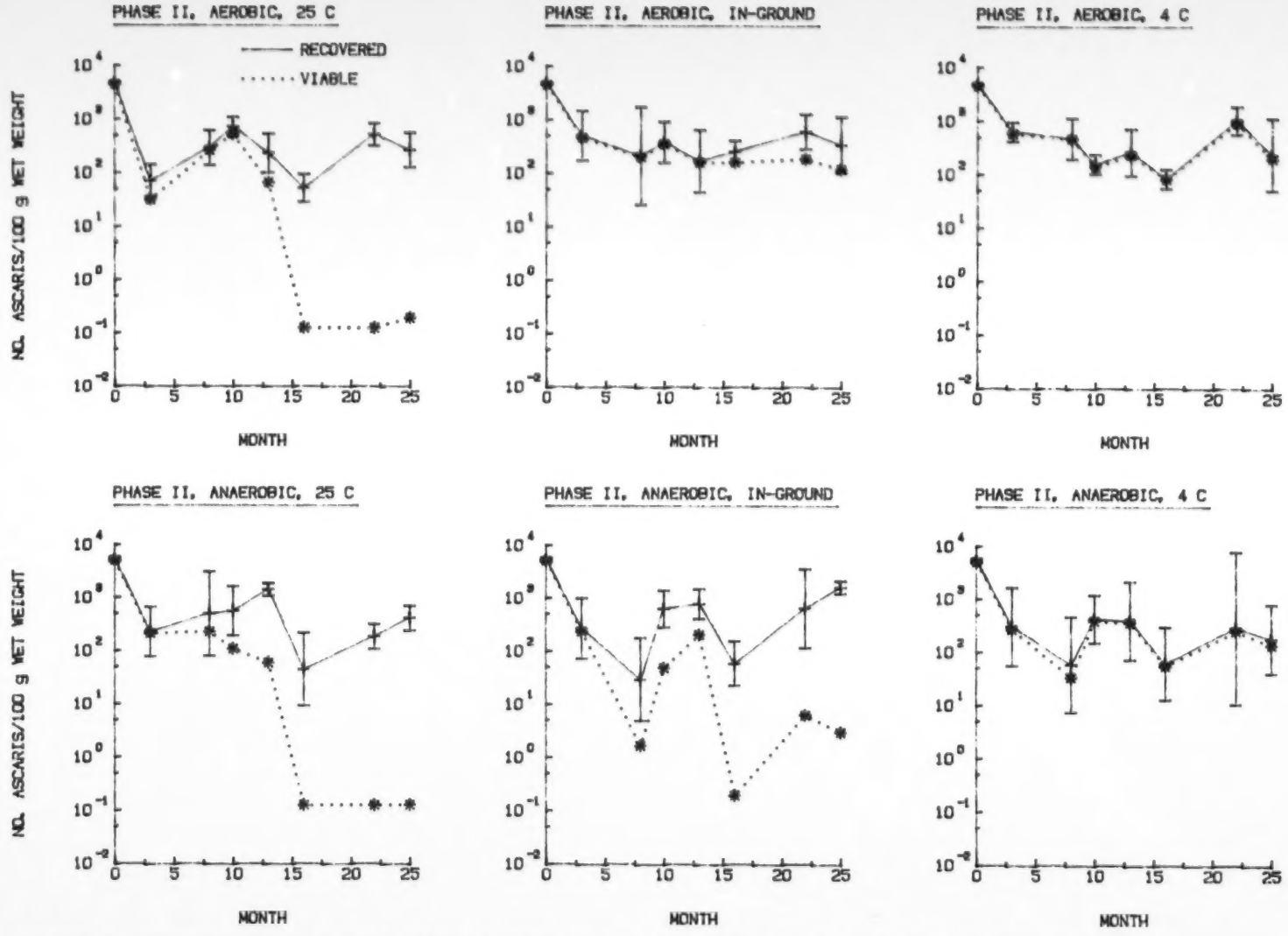

Figure 6. Recovered and viable Ascaris eggs in Phase II aerobic and anaerobic sludge samples.

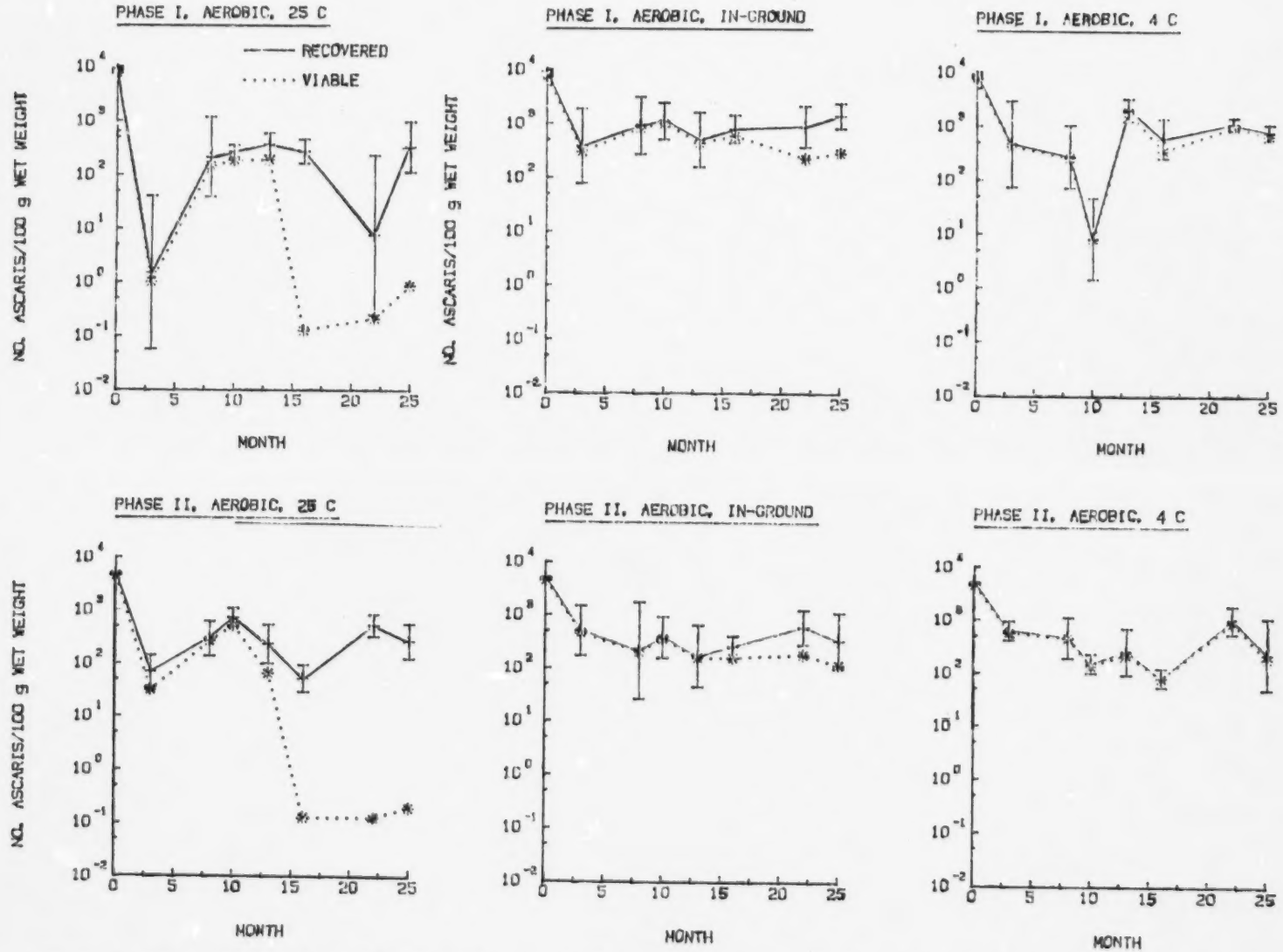

Figure 7. Recovered and viable Ascaris eggs, Phases I and II aerobic sludge samples.

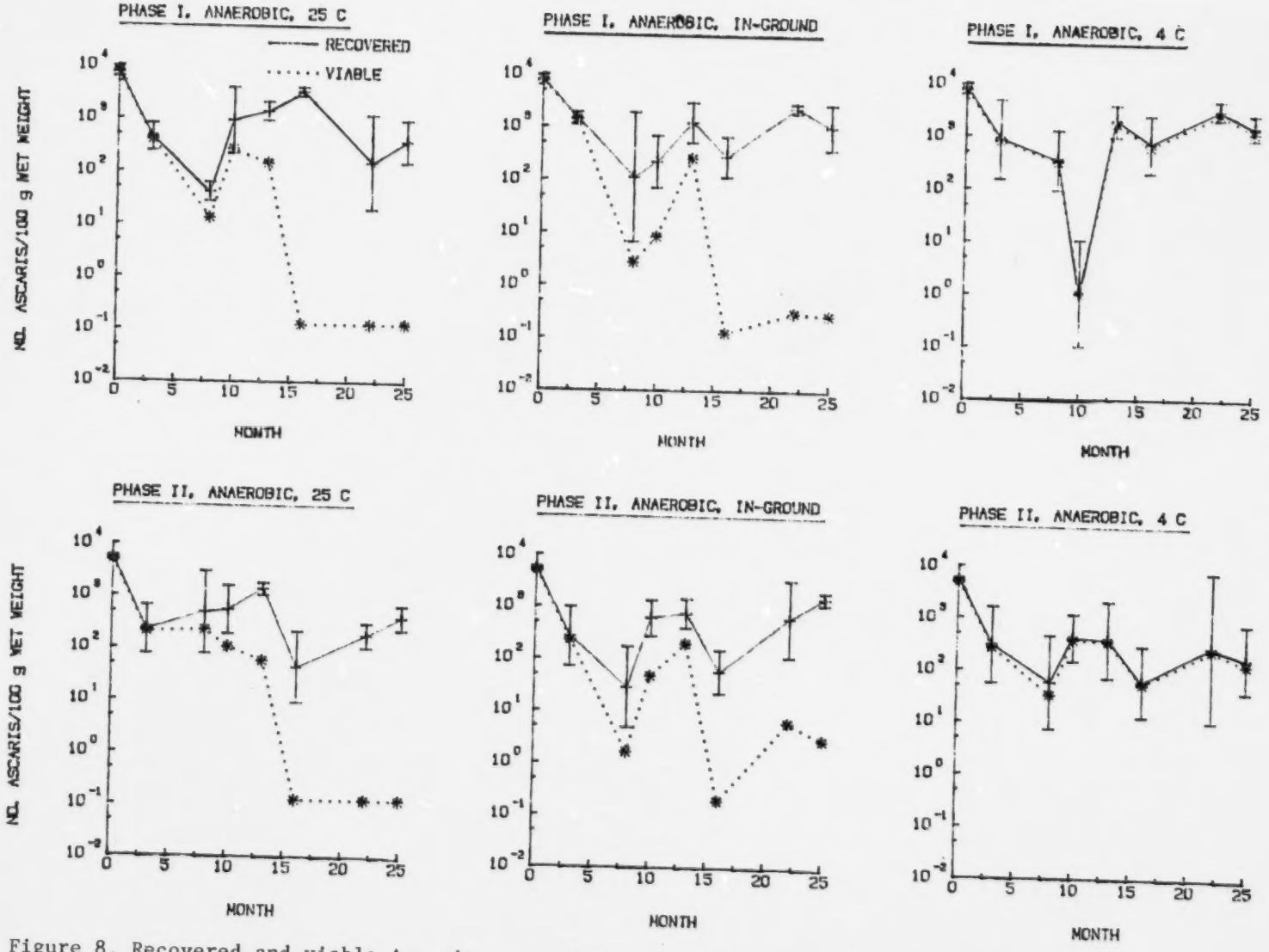

Figure 8. Recovered and viable Ascaris eggs, Phases I and II anaerobic sludge samples.

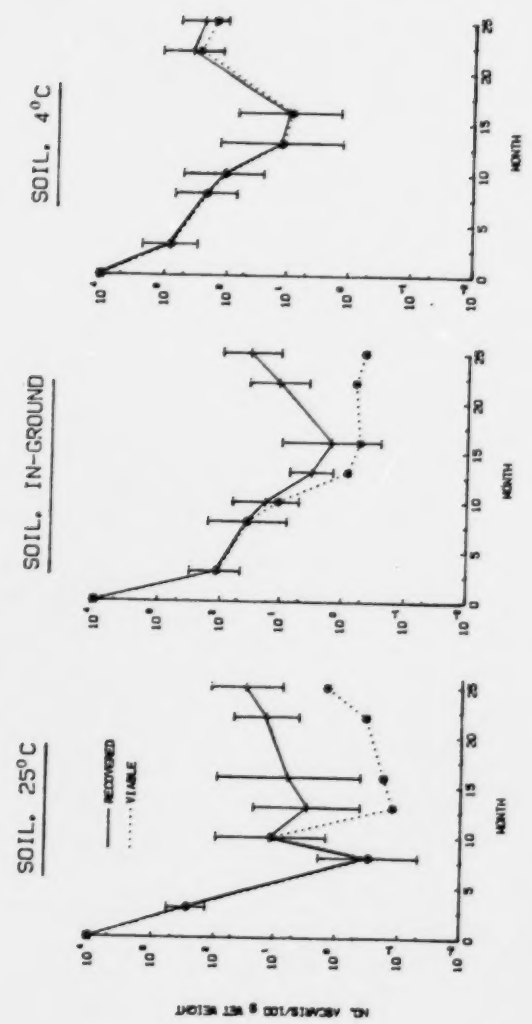

Figure 9. Recovered and viable **Ascaris** eggs, soil samples.

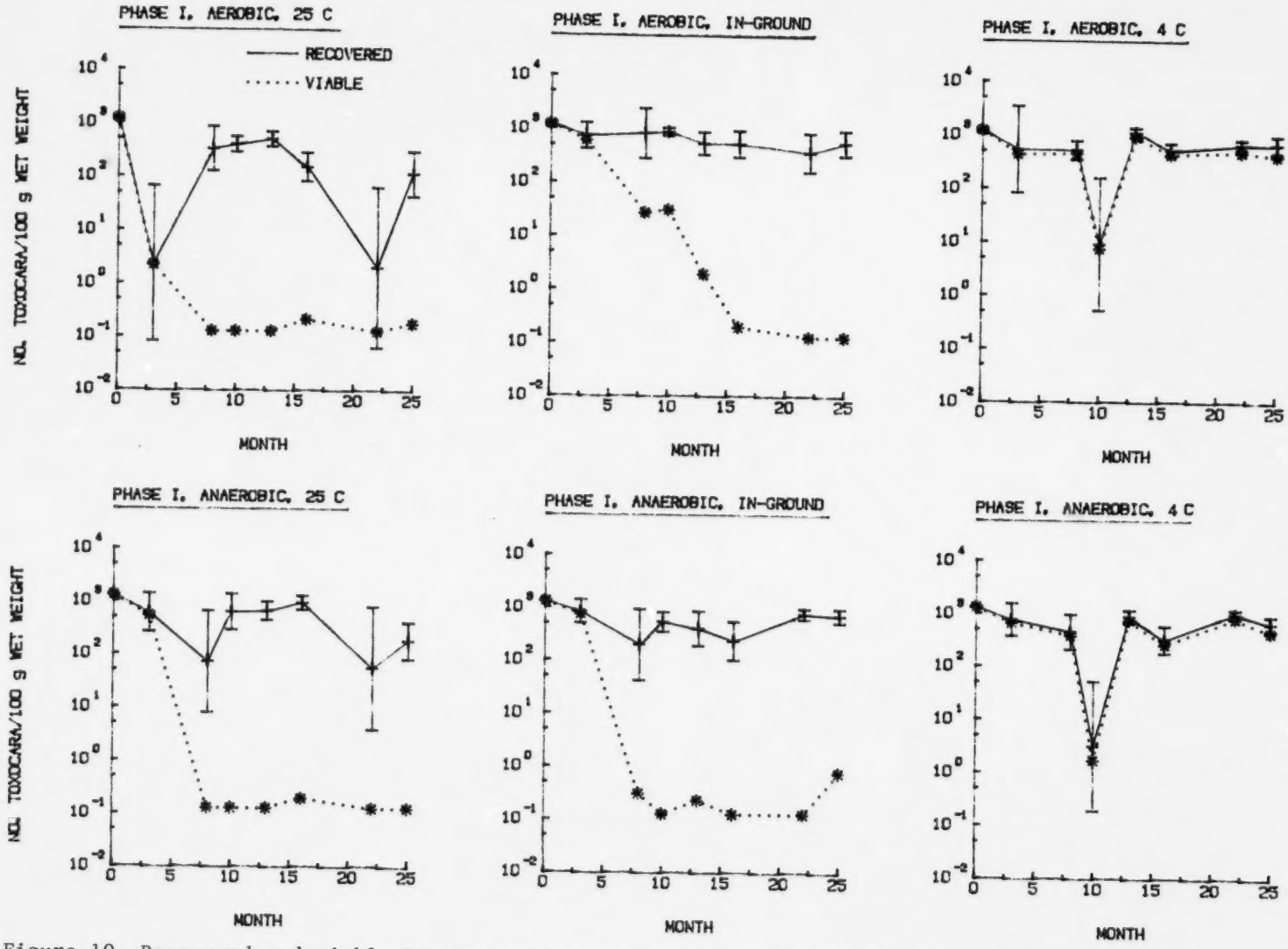

Figure 10. Recovered and viable Toxocara eggs in Phase I aerobic and anaerobic sludge samples.

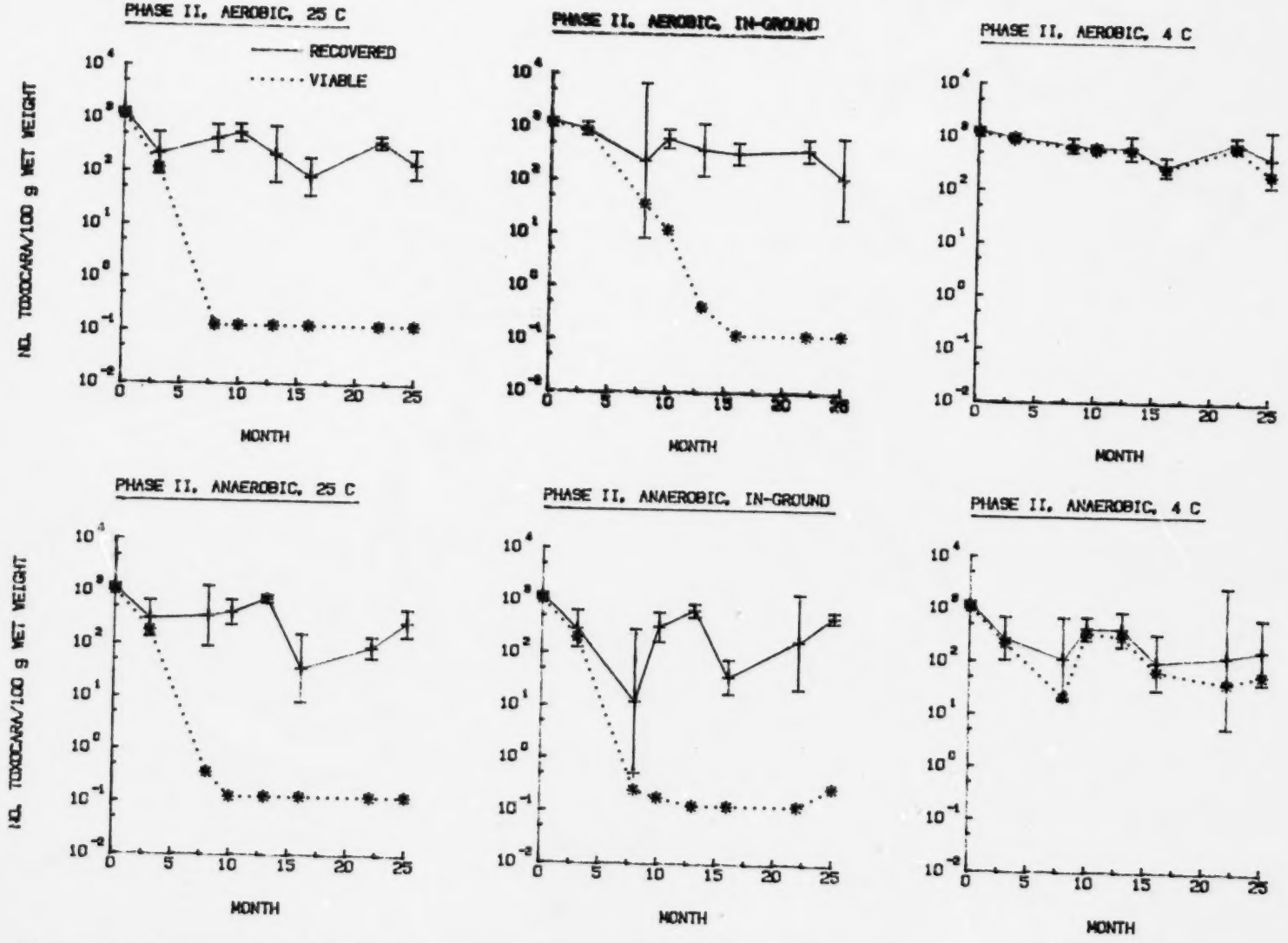

Figure 11. Recovered and viable Toxocara eggs in Phase II aerobic and anaerobic sludge samples..

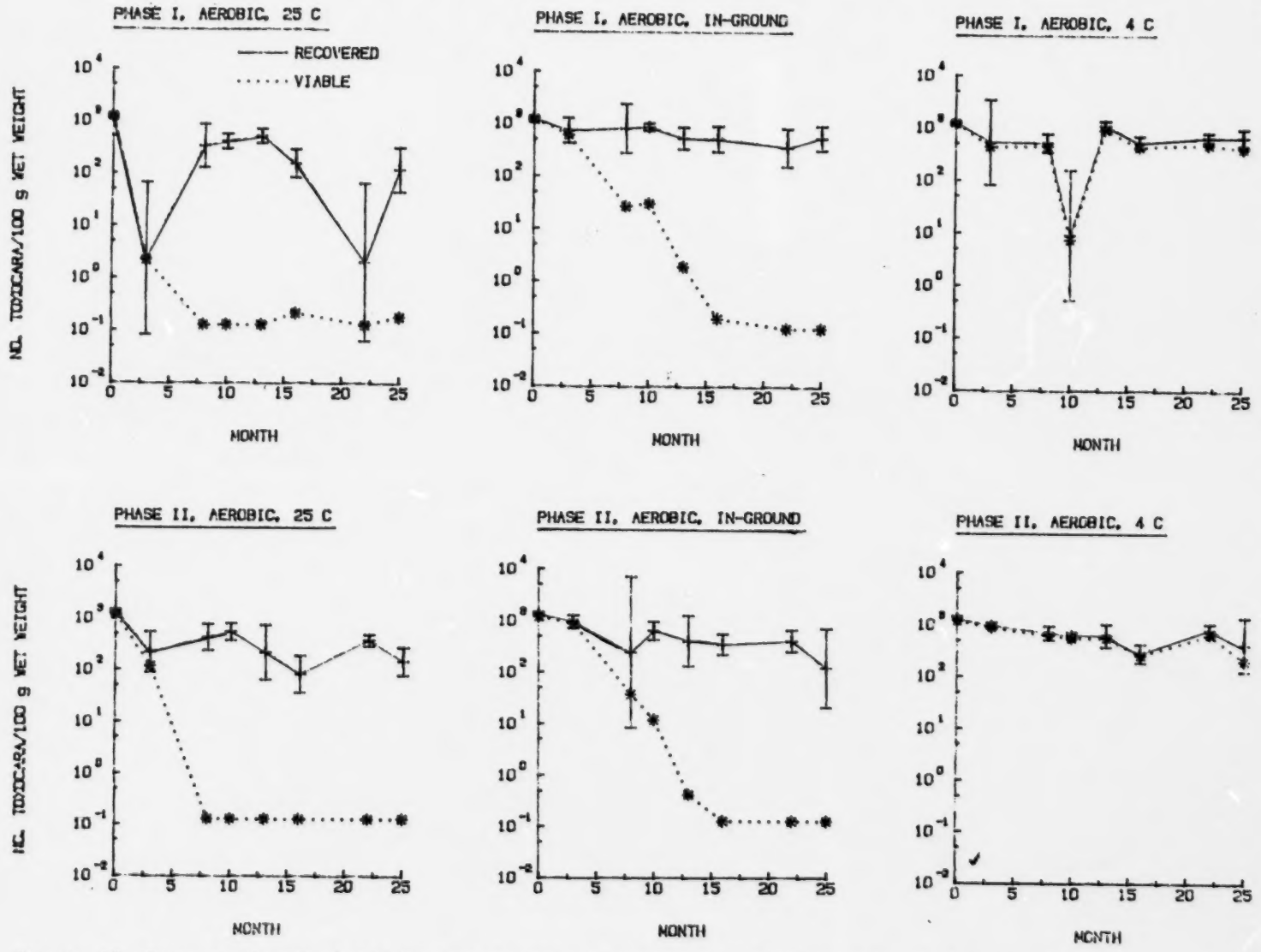

Figure 12. Recovered and viable Toxocara eggs in Phases I and II aerobic sludge samples..

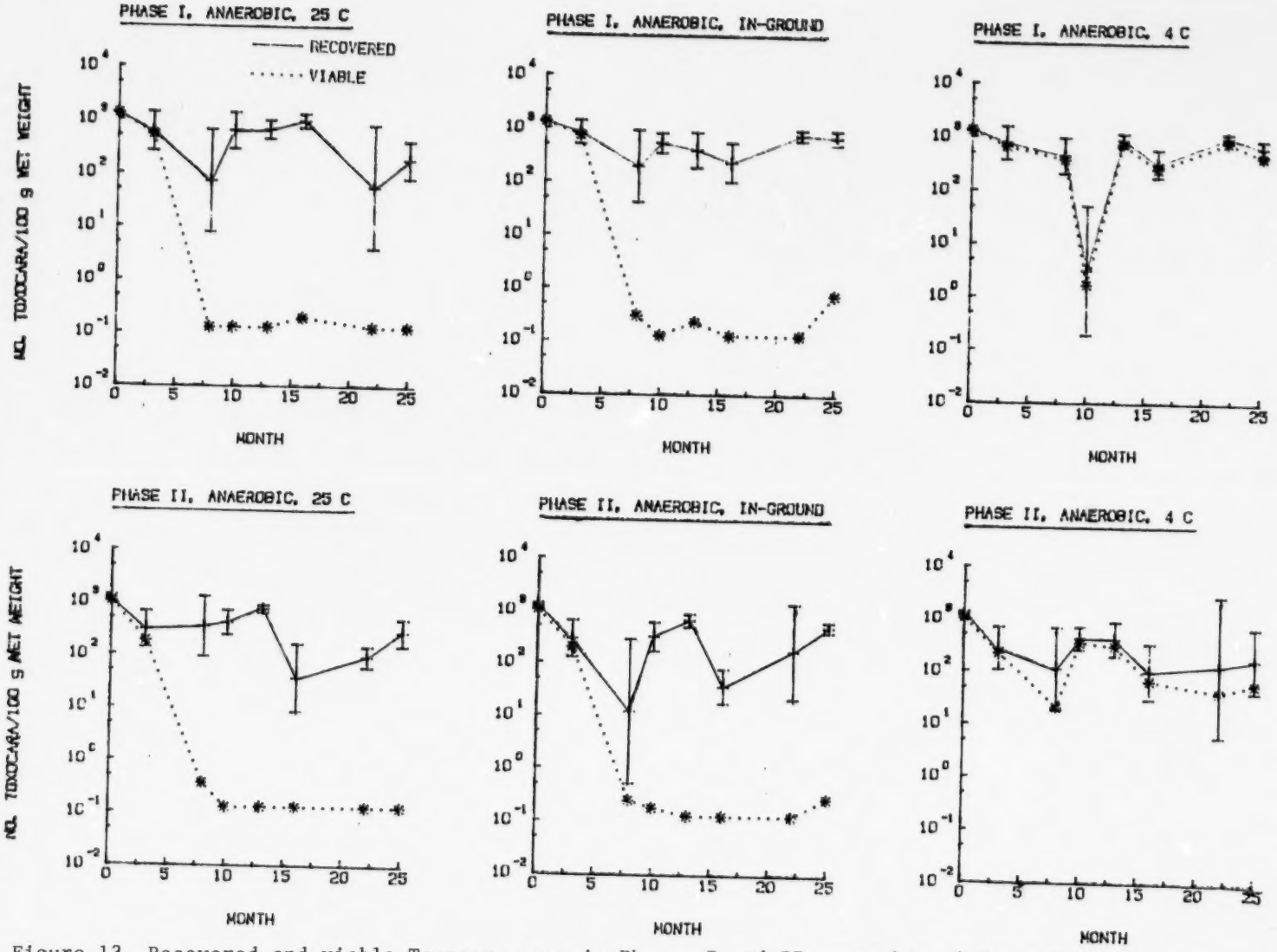

Figure 13. Recovered and viable Toxocara eggs in Phases I and II anaerobic sludge samples.

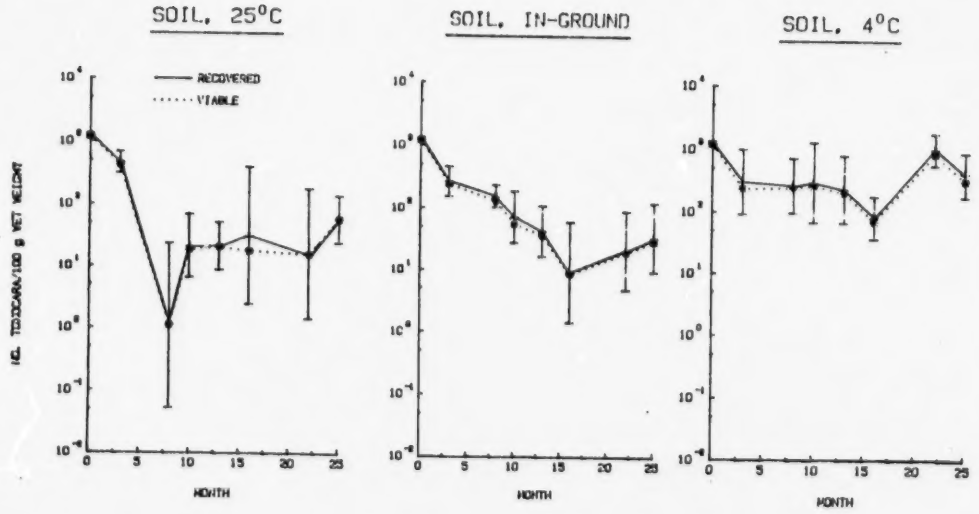

Figure 14. Recovered and viable Toxocara eggs, soil samples.

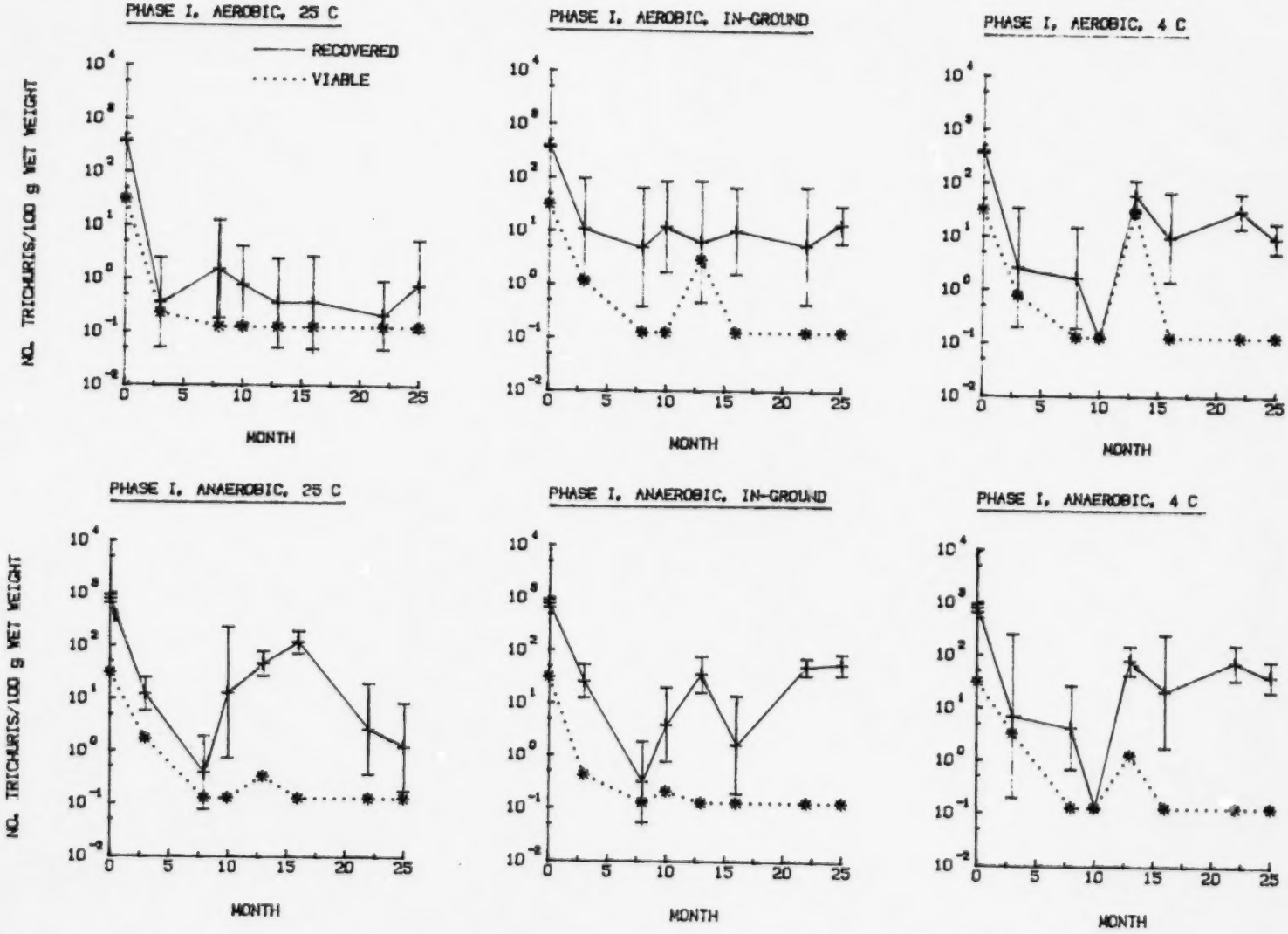

Figure 15. Recovered and viable Trichuris eggs in Phase I aerobic and anaerobic sludge samples.

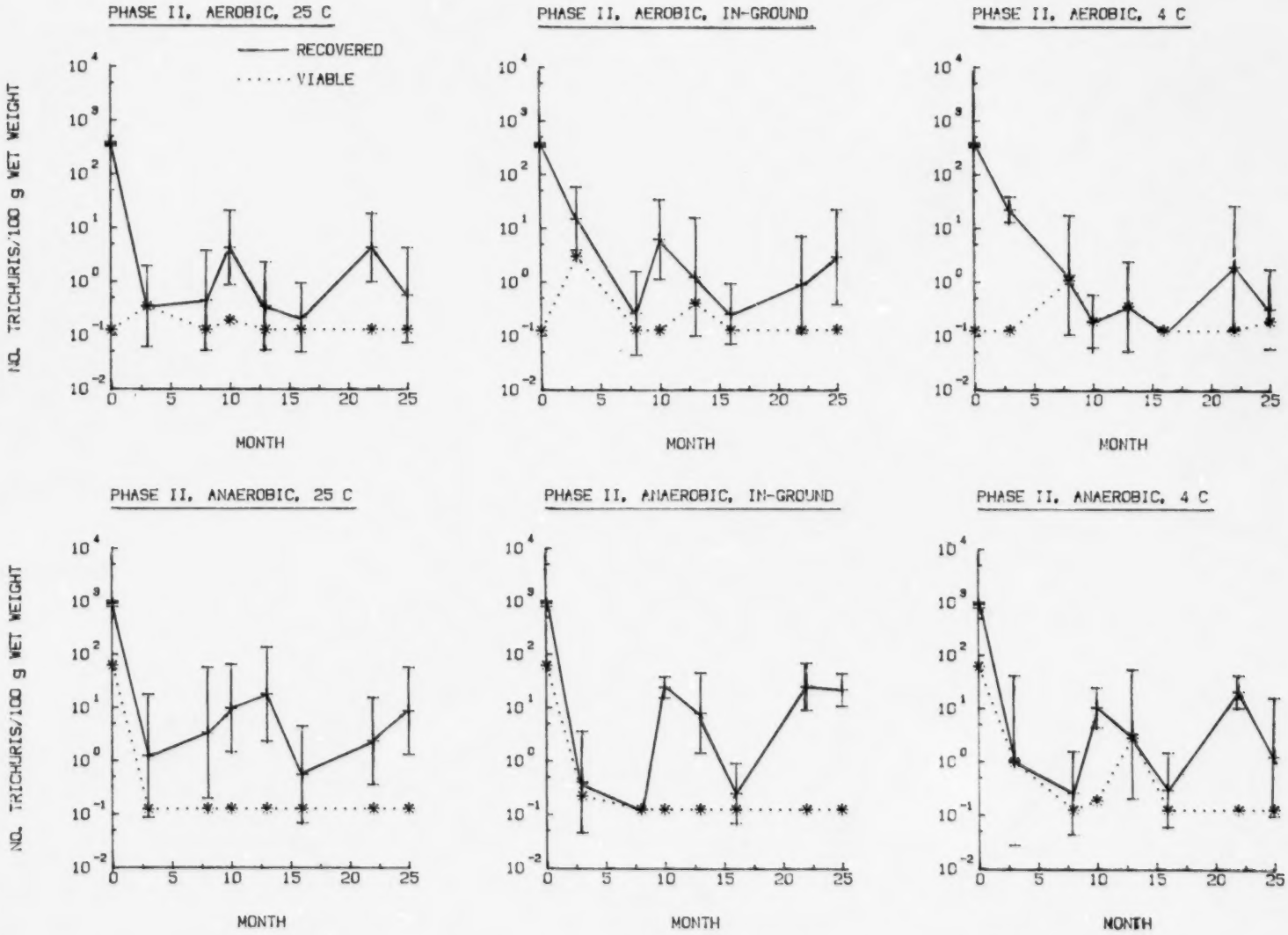

Figure 16. Recovered and viable Trichuris eggs in Phase II aerobic and anaerobic sludge samples.

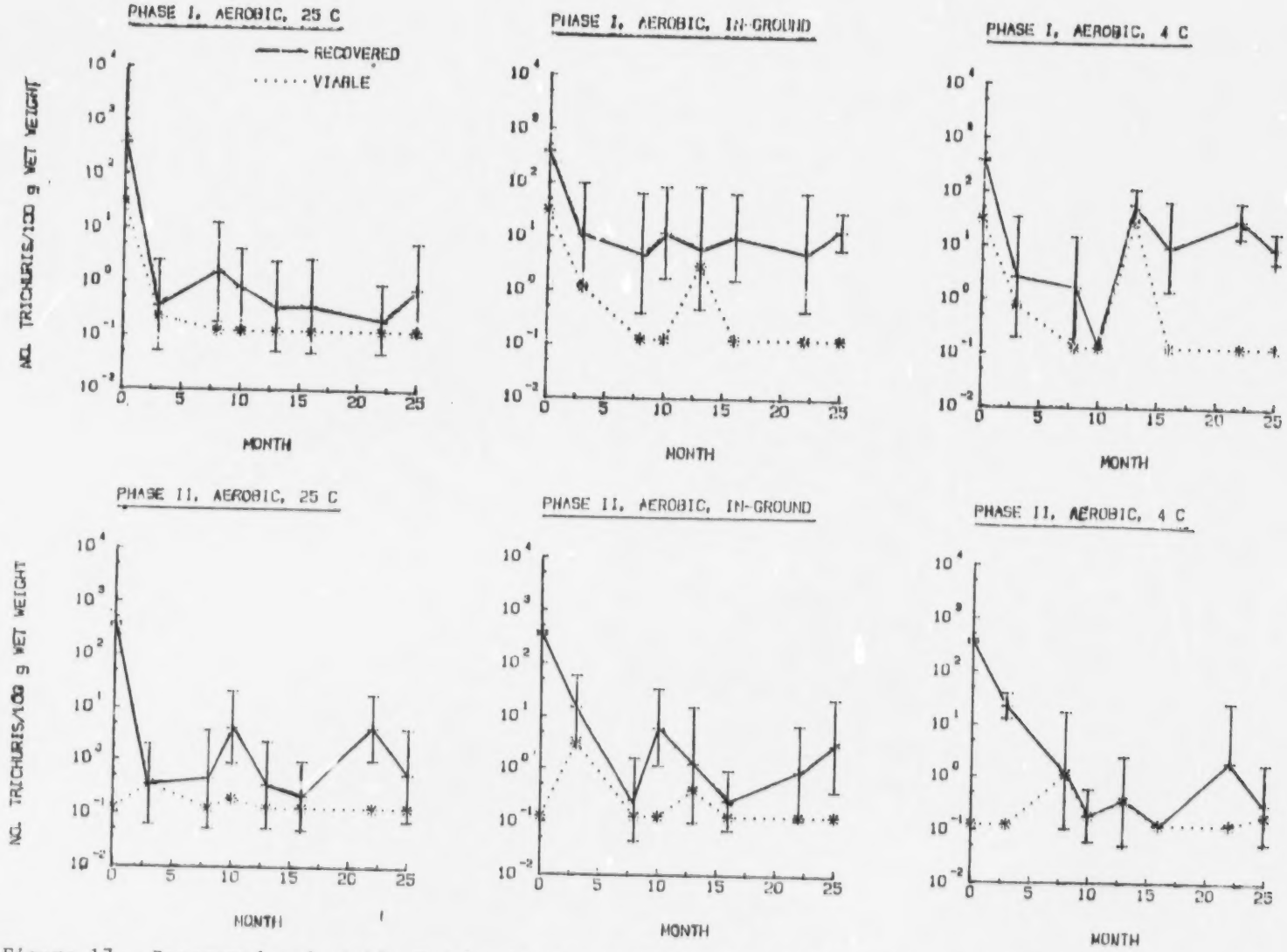

Figure 17. Recovered and viable Trichuris eggs in Phases I and II aerobic sludge samples.

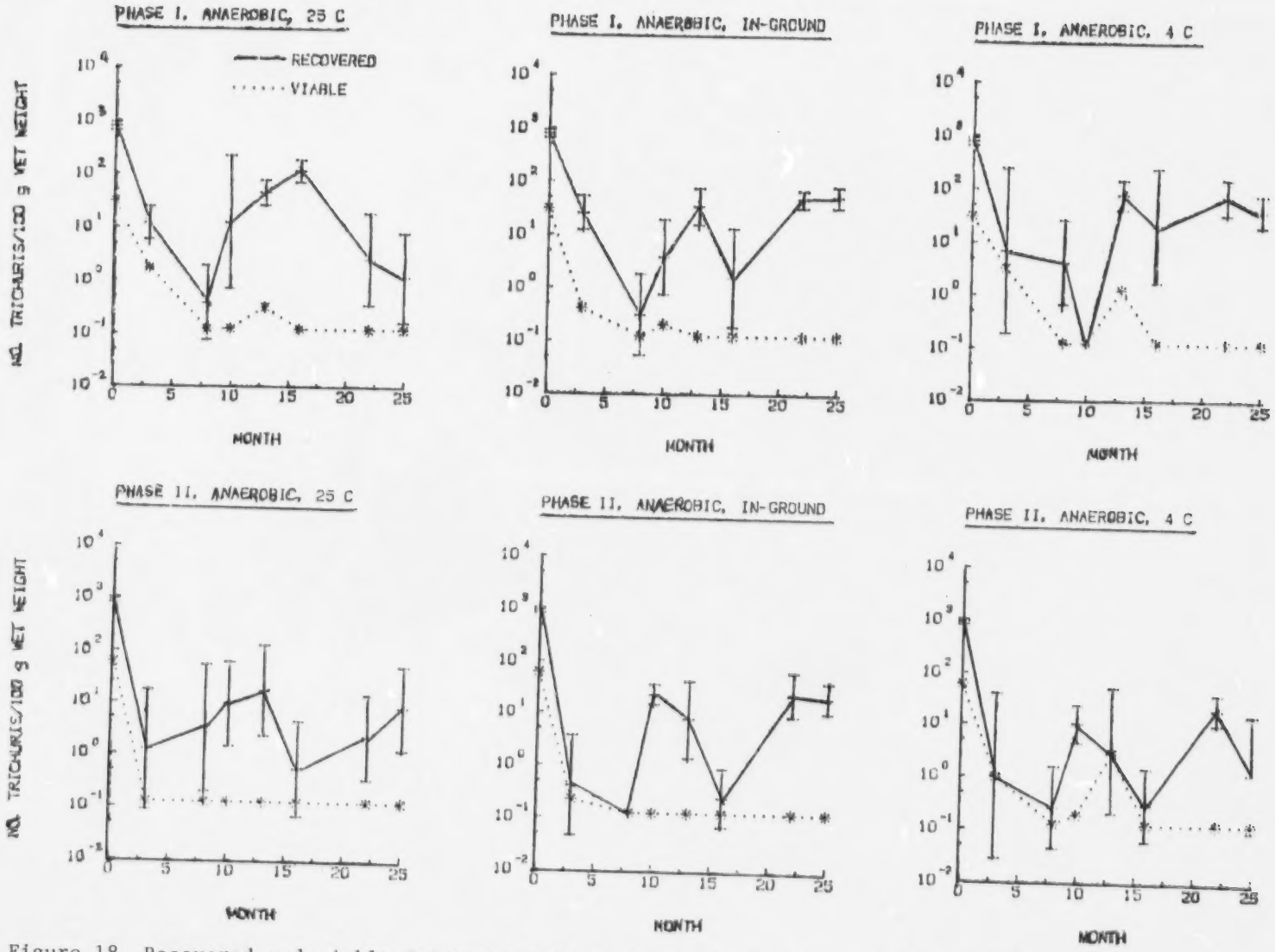

Figure 18. Recovered and viable Trichuris eggs in Phases I and II anaerobic sludge samples.

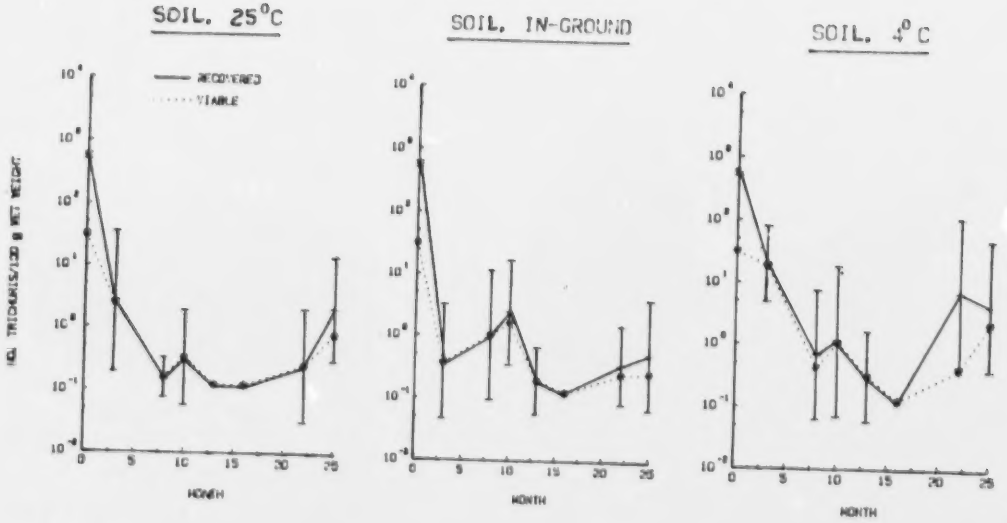

Figure 19. Recovered and viable Trichuris eggs, soil samples.

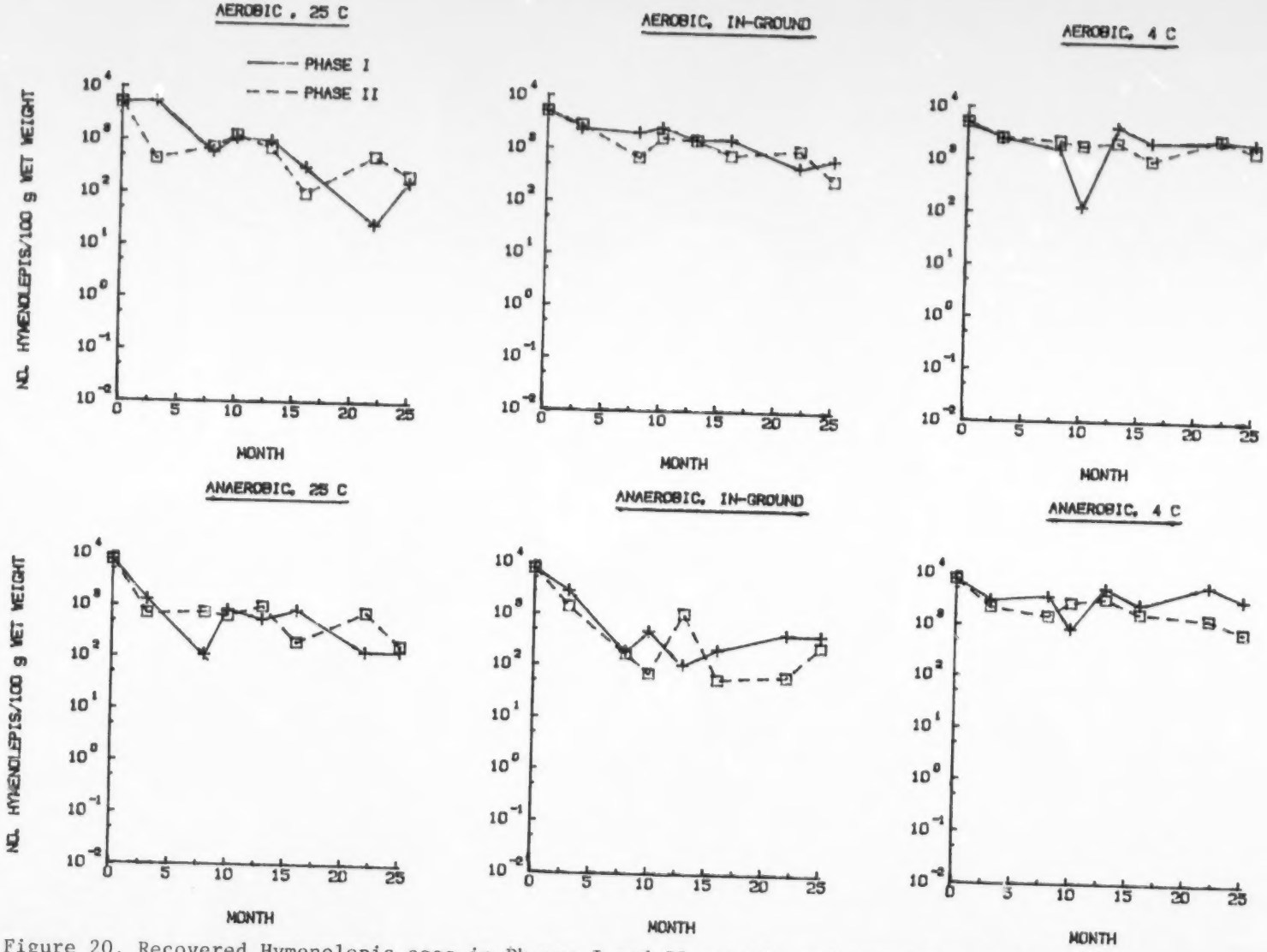

Figure 20. Recovered Hymenolepis eggs in Phases I and II aerobic and anaerobic sludge samples (tic standard deviation marks not included).

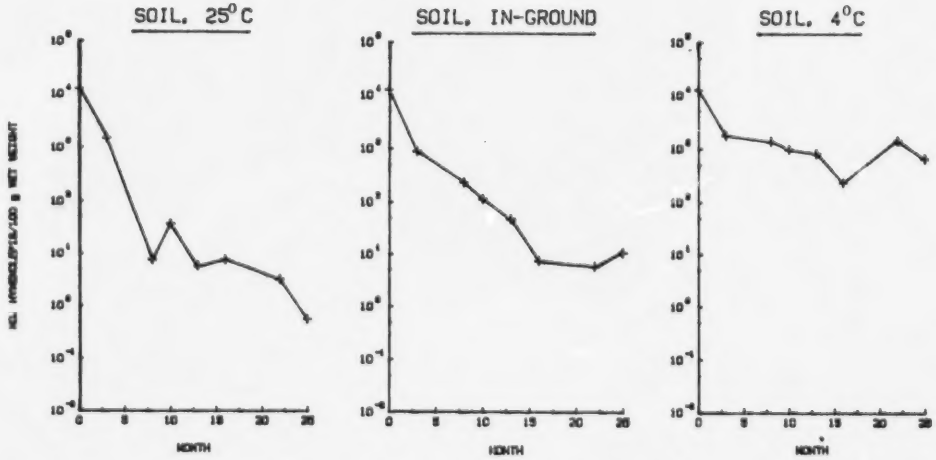

Figure 21. Recovered Hymenolepis eggs, soil samples.
(tic standard deviation marks not included)

sludge. Perhaps there is some agent (e.g. ammonia gas) produced in the anaerobic digested sludge that inactivates these parasite eggs sooner. The significant effect on Trichuris eggs is in the recovery but not in the viability of these eggs, in aerobic versus anaerobic digested sludges. Figures 15 and 16 and appendix Table A-13 suggest a greater recovery initially from anaerobic digested sludges as compared to aerobic digested sludges under the 25°C storage conditions. There is some significance in the recovery of Hymenolepis eggs in the ground storage samples. Figure 20 suggests less recovery of Hymenolepis eggs in anaerobic as compared to aerobic digested sludge stored in the ground.

The effect of how the test parasite eggs were added to the digested sludges; that is, before and during digestion or after the aerobic and anaerobic digestion was completed is also shown in the ANOVA (Phase I versus Phase II digested sludge), Table 3. Overall, there is little effect on the method of adding the test parasite eggs to the sludges. The exceptions are Ascaris eggs recovered; the significant effect is difficult to pinpoint in Figures 7 and 8; and for Trichuris eggs in Phase I and Phase II aerobic sludges (Figures 15 and 16).

Including the soil data in the ANOVA (Table 4) resulted in a significant effect for Ascaris eggs recovered in aerobic as compared to anaerobic digested sludge. The degree of freedom is still one because the soil samples were considered similar to adding Ascaris eggs to aerobic digested sludge. Generally, it was observed that recovery of the parasite eggs from soils was less than similar eggs recovered from the digested sludges. The other change was that recovery of Hymenolepis eggs was no longer significant after the inclusion of the soil data.

The degree of freedom changed from one to two for the comparison of Phase I/Phase II digested sludges and soil treatment. In every comparison of the test parasites, significant effects increased and for several comparisons actually changed the effect from no significance to a significant effect. The lower recovery of the test eggs from the soils are probably the major cause for the change in significance; which also affects the significance of viable eggs observed.

Further discussion relating to each of the test parasites and special studies follow.

RECOVERY OF ASCARIS SUUM EGGS FROM DIGESTED SLUDGES AND SOIL SAMPLES

The recovery of Ascaris eggs from digested and soil samples are shown in Figures 5 through 9 and appendix Tables A-1, A-3 and A-5.

Initially (at time = 0) more eggs were recovered from sludges to which Ascaris eggs had been added after digestion (Phase I) than from the sludges that had the eggs added before and during digestion (Phase II). This difference was probably due to the destructive effects of digestion processes on the Ascaris eggs. Upon storage, there appears to be a slightly lower recovery of Ascaris eggs from aerobic as compared to anaerobic digested sludge.

The amount of Ascaris eggs recovered from soil samples (Figure 9) was consistently less than recovered from the digested sludges. This recovery difference was about one log. Whether this recovery difference is a result of greater Ascaris egg destruction in soils, or that the recovery of Ascaris eggs from soils is not as efficient as from the digested sludges is unknown.

VIABILITY OF ASCARIS EGGS

The data on the effect of storage conditions on the viability of Ascaris eggs are shown in Figures 5 through 9 and Appendix Tables A-2, A-4 and A-6. Several important factors stand out: (1) The decrease in the viability of Ascaris eggs was significantly more rapid during the 25°C storage as compared to in-ground and 4°C storage. (2) The decrease in the viability of Ascaris eggs began sooner in anaerobic as compared to aerobic digested sludge for the in-ground storage samples. (3) There was little decrease in the viability of Ascaris eggs in the 4°C storage samples. (4) There appears to be no significant difference resulting from the method of adding Ascaris eggs to the digested sludges. The inclusion of the soil data, however, resulted in a significant difference, and this apparent difference is probably the result of lower recovery of Ascaris eggs from the soil samples.

25°C Stored Samples

All Ascaris eggs recovered from anaerobic digested sludge appeared to be nonviable and noninfective after about 16 months storage (Figures 5 and 6, Appendix Table A-2), whereas about 25 months were required to mostly inactivate the Ascaris eggs recovered from aerobic sludges. This difference in the inactivation of Ascaris eggs could be due to the presence of a parasiticidal substance (e.g. ammonia) in the anaerobic digested sludge. Based on these results, about 2 years of storage under a warm climatic environment are needed to mostly inactivate the digester resistant Ascaris eggs.

The viability of most eggs recovered from the soil also decreased rapidly after 10 months of storage but some of the eggs still maintained their viability through the end of the study (Figure 9; Appendix Table A-2). There was no indication of infectivity after 10 months of storage at 25°C.

In-Ground Stored Samples

Ascaris eggs recovered from anaerobically digested sludges (Phase I and Phase II) showed a rapid decrease in their viability after 16 months in-ground storage (Figures 5 and 6, Appendix Table A-4). Eggs in aerobically digested sludges (Phase I and Phase II) showed little reduction in their viability during their first 16 months of storage. This was followed by a gradual decrease in viability over the next year, until after the 30-month sampling period at which time none of the recovered eggs appeared to be viable, though at 33-months storage there was some evidence of infectivity in Phase I aerobically digested sludge (Table 5). Eggs stored in soil did show a reduction in their viability but at the end of the study (33 months storage), some of the eggs recovered were still viable (Figure 9, Table A-4).

4°C Stored Samples

After about 2 years of storage at 4°C, there was only a slight decrease in the viability of Ascaris suum eggs recovered from sludge and soil samples (Figure 5 through 9 and appendix Table A-6).

RECOVERY OF TOXOCARA CANIS EGGS FROM DIGESTED SLUDGES AND SOIL SAMPLES

The recovery of Toxocara eggs from the digested sludges and soil samples are shown in Figures 10 through 14 and appendix Tables A-7, A-9 and A-11. In comparing the ANOVA that includes the soil data (Table 4), a significant effect was noted in the recovery of Toxocara eggs. The recovery of Toxocara eggs from the soil samples was about one log less than their recovery from the digested sludges. Again, whether this recovery difference could be the result of greater Toxocara egg destruction in soils, or that the recovery of Toxocara eggs from soils is not as efficient as from the digested sludges is unknown.

VIABILITY OF TOXOCARA EGGS

The data on the effect of storage conditions on the viability of Toxocara eggs are shown in Figures 10 through 14 and appendix Tables A-8, A-10 and A-12.

25°C Stored Samples

None of the recovered Toxocara eggs from the digested sludges were viable following the first 8 months in storage at 25°C. Toxocara isolated from the soil samples, however, were not affected. Further, there was little reduction in the viability of Toxocara eggs in the soil samples under any of the three storage conditions tested. This effect is in sharp contrast to the viability data for the other test parasite eggs. Why Toxocara eggs remained viable in soils is uncertain, though it appears that the digester sludges may have an effect on the viability of Toxocara eggs.

The infectivity of Toxocara eggs recovered from digested sludges stored at 25°C was negative after 10 months (Table 6). Toxocara eggs recovered from the soil samples remained infective for over 13 months but did not show further infectivity even though the viability of Toxocara eggs persisted.

In-Ground Stored Samples

Toxocara eggs in the digested sludges became mostly non-viable after one year of storage in the ground. This effect was observed sooner in anaerobic digested sludge; eight months versus 13 months in aerobic digested sludge. Also, there was no significant difference in Toxocara egg viability between Phase I and Phase II spiking of the digested sludges (Figures 12 and 13 and Table A-10).

Toxocara eggs isolated from the soil samples were not affected. In fact, they maintained their initial level of viability throughout the

entire 33 month period (Figure 14, appendix Table A-10). These results, along with those from the 25°C samples, may indicate the presence of a substance (or substances) toxic to the Toxocara canis eggs in the sludge samples that is absent in the soil samples.

4°C Stored Samples

The dramatic loss of viability of Toxocara eggs seen in the 25°C and the in ground stored samples did not occur in the 4°C digested sludge samples. Again, there was no decrease in the viability of eggs recovered from the soil samples. The putative toxic material(s) present in sludges may have had less effect at lower temperatures (Figure 12, 13, and 14; appendix Table A-12) as compared to its effect at 25°C or when stored in the ground.

RECOVERY OF TRICHURIS EGGS FROM DIGESTED SLUDGES AND SOIL SAMPLES

The recovery of Trichuris eggs from the digested sludges and soil samples is shown in Figures 15 through 19 and appendix Tables A-13, A-15 and A-17.

Unlike the recovery of Ascaris and Toxocara eggs, the ANOVA's in Tables 3 and 4 indicate significant effects between aerobic and anaerobic sludge digestion and between Phase I/Phase II and soil spiking with the Trichuris eggs. Review of Figures 15 through 19 suggest possible differences, but these differences are difficult to pinpoint because of the low and erratic numbers of Trichuris eggs recovered.

VIABILITY OF TRICHURIS EGGS

It is difficult to comment on the viability of the Trichuris eggs recovered because, as mentioned earlier, the recovery rates for these eggs were low and erratic and large standard deviations were observed. Therefore, only a most general statement as to the viability of these eggs is possible. After 13 months of storage in sludges at 4°C, at 25°C or in the ground, most of the Trichuris eggs were no longer viable (Figures 15 through 19 and appendix Tables A-14, A-16 and A-18). Eggs stored in soil, however, appear to retain their viabilities for at least 25 months.

RECOVERY OF HYMENOLEPIS DIMINUTA EGGS FROM DIGESTED SLUDGES AND SOIL SAMPLES

As mentioned previously, no data are available on the survival of Hymenolepis diminuta eggs because the viability of these eggs was compromised before they were added to the samples. Storage over several months (during the accumulation of eggs) even at 4°C, was sufficient to render them nonviable. However, although these eggs were dead, they remained intact and identifiable even after digestion and storage for the extent of this study.

After 3 months in storage at 25°C and in the ground, the rate of recovery of Hymenolepis eggs from all samples dropped sharply. The eggs

were being destroyed because visual observations of the eggs recovered from stored samples indicated that they had deteriorated. The percent of eggs recovered from the samples stored at 4°C also decreased with time, but the rate was slower. The lower temperature probably slowed down the process(es) involved in the destruction of these non-viable eggs (Figures 20 and 21 and appendix Tables A-19 through A-21).

INFECTIVITY STUDIES

During the discussion of the viability of the test parasites reference was made to the infectivity studies using laboratory rats as the test host. A summary of the viability and infectivity of the recovered Ascaris and Toxocara eggs is shown in Tables 5 and 6.

Phase II sludges are the most analogous to a real situation in which parasite eggs are introduced to sludge during digestion processes. The results of Phase I in which eggs were added after sludges had been digested and before storage occurred were used to test whether or not the digestion processes of Phase II sludges had any effect on the recovery, viability and infectivity of the parasite eggs tested, that is, the eggs in Phase I sludges were not subjected to the digestion processes. Soil controls were used to test whether or not the composition of the sludges themselves had any effect on the inactivation, viability and infectivity of the eggs. It was noted that although eggs often appeared viable, they did not necessarily produce infections in test rats.

As indicated in the RESULTS SECTION, the data on infectivity are qualitative and not quantitative because we scored only negatives versus positive infections. These data were obtained by intubations of different numbers of recovered eggs and have no meaning since factors such as dose could influence the ability of a parasite to infect an animal. A positive (+) denotes the presence of one or more larvae in one or more of the test rat organs examined. A negative (-) indicates that no larval forms of the parasite were found in the tissues examined. In some instances, hemorrhaging was noted in the lungs of the sacrificed rat, this alone was not considered evidence of parasite infection and were scored as negatives unless larvae were also found.

Infectivity of Ascaris Eggs

Anaerobic digested sludges stored at 25°C for at least 16 months proved to be the most effective set of conditions for the inactivation of Ascaris eggs. Further, the anaerobic digestion process itself seems to increase the resistance of Ascaris eggs to storage conditions. Ascaris eggs added to anaerobically digested sludge after digestion (Phase I) lost their infection capability after only 3 months storage at 25°C; whereas it took 16 months for these eggs to lose their infective capability when digested along with the sludge (Phase II). The same observation holds true for eggs that were subjected to aerobic digestion. Eggs added to aerobically digested sludge lost their infectivity by 16 months; eggs added during the aerobic digestion of the sludge took 25 months to lose infectivity.

Table 5. Viability and infectivity of recovered Ascaris eggs.

Months in storage	3		8		10		13		16	
	V*	I+	V	I	V	I	V	I	V	I
Anaerobic I										
25°C	+	-	+	-	+	-	+	-	-	-
4°C	+	QNS	+	+	+	QNS	+	+	+	+
ground	+	-	+	-	+	-	+	+	-	-
Aerobic I										
25°C	+	QNS	+	+	+	+	+	+	-	-
4°C	+	+	+	+	+	QNS	+	+	+	+
ground	+	+	+	+	+	+	+	+	+	+
Anaerobic II										
25°C	+	QNS	+	-	+	+	+	+	-	-
4°C	+	+	+	-	+	-	+	+	+	-
ground	+	-	+	+	+	+	+	+	+	-
Aerobic II										
25°C	+	-	+	+	+	+	+	+	-	+
4°C	+	QNS	+	+	+	-	+	+	+	+
ground	+	+	+	+	+	+	+	+	+	+
Soil										
25°C	+	+	+	QNS	+	-	-	-	+	-
4°C	+	+	+	+	+	-	+	-	+	+
ground	+	-	+	+	+	-	+	-	+	+

*Viability
+Infectivity
QNS = Quantity not sufficient for analysis

(continued)

Table 5. (Continued)

Months in storage	19		22		25		30		33	
	V*	I+	V	I	V	I	V	I	V	I
Anaerobic I										
25°C	-	-	-	-	-	-	#		#	
4°C	+	+	+	+	+	-	#		#	
ground	-	-	+	-	+	-	#		#	
Aerobic I										
25°C	-	-	+	-	+	-	-	-	-	-
4°C	+	+	+	+	+	-	#		#	
ground	+	+	+	+	+	-	+	-	-	+
Anaerobic II										
25°C	-	-	-	-	-	-	#		#	
4°C	+	+	+	+	+	+	#		#	
ground	+	+	+	+	+	-	+	-	-	-
Aerobic II										
25°C	-	-	-	+	+	-	+	-	-	-
4°C	+	-	+	+	+	-	#		#	
ground	+	-	+	+	+	-	-	-	-	-
Soil										
25°C	-	-	+	-	+	-	+	-	+	-
4°C	+	-	+	+	+	+	#		#	
ground	-	-	+	-	+	-	+	-	+	-

*Viability
+Infectivity
Data not requested

Table 6. Viability and infectivity of recovered Toxocara canis eggs.

Months in storage	3		8		10		13		16	
	V*	I+	V	I	V	I	V	I	V	I
Anaerobic I										
25°C	+	+	-	-	-	-	-	-	-	-
4°C	+	QNS	+	+	+	-	+	+	+	+
ground	+	+	-	-	-	-	-	-	-	-
Aerobic I										
25°C	+	QNS	-	-	-	-	-	-	-	-
4°C	+	+	+	+	+	QNS	+	+	+	+
ground	+	+	+	+	+	+	-	-	-	-
Anaerobic II										
25°C	+	QNS	-	+	-	-	-	-	-	-
4°C	+	+	+	+	+	+	+	+	+	+
ground	+	+	+	-	-	-	-	-	-	-
Aerobic II										
25°C	+	+	-	-	-	-	-	-	-	-
4°C	+	QNS	+	+	+	+	+	+	+	+
ground	+	+	+	-	+	+	+	-	-	+
Soil										
25°C	+	+	+	QNS	+	+	+	+	+	-
4°C	+	+	+	+	+	+	+	+	+	+
ground	+	+	+	+	+	+	+	-	+	+

*Viability
+Infectivity
QNS = Quantity not sufficient for analysis

(continued)

Table 6. (Continued)

Months in storage	19		22		25		30		33	
	V*	I+	V	I	V	I	V	I	V	I
Anaerobic I										
25°C	-	-	-	-	-	-	#		#	
4°C	+	+	+	+	+	+	#		#	
ground	-	-	-	-	+	+	#		#	
Aerobic I										
25°C	-	-	-	-	+	-	-	-	-	-
4°C	+	+	+	+	+	+	#		#	
ground	-	-	-	-	-	-	-	-	-	-
Anaerobic II										
25°C	-	-	-	-	-	-	#		#	
4°C	+	+	+	+	+	+	#		#	
ground	-	-	-	-	+	-	-	-	-	-
Aerobic II										
25°C	-	-	-	-	-	-	-	-	-	-
4°C	+	+	+	+	+	+	#		#	
ground	+	-	-	-	-	-	-	-	-	-
Soil										
25°C	+	-	+	-	+	-	+	-	+	-
4°C	+	-	+	+	+	+	#		#	
ground	+	-	+	-	+	+	+	-	+	+

*Viability
+Infectivity
Data not requested

Infective Ascaris eggs were recovered after 22 months from both aerobic and anaerobic digested sludge samples (Phase II) that were placed in the ground under fluctuating temperatures. Eggs from sludges stored at 4°C remained infective for nearly the entire period for which data is available (25 months). By 10 months, eggs recovered from soil controls stored at 25°C had lost their infectivity. This loss of infectivity of Ascaris eggs was much earlier than eggs stored in either anaerobic or aerobic digested sludges to which eggs were added during the digestion process (Phase II). Exposure to sludge digestion itself again appeared to increase the ability of the Ascaris eggs thus processed to remain infective for a longer period of storage.

Infectivity of Toxocara Eggs

Loss of infectivity of Toxocara eggs in Phase II sludges occurred slightly sooner in aerobically digested sludges (by 8 months) as compared to anaerobically digested sludges (by 10 months) when stored at 25°C. There was very little difference between the length of time required to render Toxocara eggs noninfective when comparing storage in Phase I with Phase II sludges. Unlike Ascaris eggs, Toxocara eggs infectivity did not seem to increase after the sludge digestion. Rather, anaerobic and aerobic digestion rendered these eggs more susceptible to loss of infectivity because eggs stored at 25°C in soil took 16 months to lose their infectivity as compared to the 8 to 10 months in digested sludges (Phase II). This decrease in the infectivity potential of Toxocara eggs due to sludge digestion (in contrast to Ascaris eggs) is interesting because although the eggs of Toxocara were more resistant to digestion than those of Ascaris, they were more quickly inactivated during storage. Eggs recovered from all sludge and soil samples stored at 4°C remained infective throughout the period for which there is data (25 months). Toxocara eggs in samples stored in the ground lost their infectivity as early as 8 months in anaerobically digested Phase II sludges and as late as 19 months for eggs recovered from aerobically digested Phase II sludge samples. At 33 months, eggs recovered from the soil samples stored in the ground remained infective.

pH MEASUREMENTS

The pH of soil and sludge samples changed with storage time (Figures 2 through 4). There were large initial increases in the pH values of all soil and anaerobically digested sludge samples, whereas aerobically digested sludge samples showed an initial decrease in pH. Upon long term storage, the largest changes in pH were seen in soil samples stored at 25°C, aerobically digested sludge samples stored at 4°C, and anaerobically digested sludge samples stored at 25°C and in the ground. No obvious correlations were noted between the pH of samples and the recovery, viability or infectivity of parasite eggs.

STAINING WITH DYES STUDIES

Several vital stains and dyes that are excluded from live cells were capable of discriminating between viable and non-viable eggs of each species of parasite separately. Crystal violet and Nile Blue Sulfate were excluded from live and absorbed by dead eggs of Ascaris suum. Benzopurpurin, Saphranin O, Neutral Red and the methanol, ethanol, acetone:ethanol fractions of the Nile Blue Sulfate mixture were capable of distinguishing between viable and non-viable eggs of Trichuris vulpis. Benzopurpurin was taken up by dead Toxocara canis eggs and excluded from living ones, whereas Nile Blue Sulfate was taken up by viable Toxocara eggs and excluded from non-viable ones. Saphranin O, Brilliant Cresyl Blue and Neutral Red were all capable of discriminating between viable and non-viable eggs of Hymenolepis diminuta. In conclusion, no one dye was capable of distinguishing between viable and non-viable eggs of all four species of parasites (Table 7).

OXIDATION-REDUCTION POTENTIAL MEASUREMENTS

The oxidation-reduction potential (ORP) of a system is a measure of the electrochemical potential or electron availability within these systems. In a system such as sludge or soil, there may be many redox couples and the measurement of the ORP of such a system reflects the degree of oxidation or reduction of the various chemical species in that system. The measured ORP is a mixed potential that reflects an average of the potentials contributed by each of the redox couples present in the system.

Patrick and Mahapatra (19) suggest four general ranges of ORP conditions in soils. These ranges were used to characterize the ORP of the sludge and soil samples. An ORP of +400 mV indicates an oxidized state; from +400 mV to +100 mV moderately reduced; from +100 to -100 mV reduced; and -100 to -300 mV highly reduced.

Of the 96 sludge samples tested after months of storage, 60% had ORP values of +400 mV or greater indicating that these samples were oxidized (Appendix B). The remaining 40% had measured ORP values in the +400 to +100 mV range indicating that these samples were moderately reduced. Neither the method of digestion (aerobic or anaerobic) nor the storage temperature (4°C, 25°C or in the ground) of these samples seemed to correlate with their measured ORP values. This lack of correlation and the variations (oxidized to moderately reduced) in ORP values within a given sample and storage condition indicate that there may be some variations in the biological activity within individual samples stored in separate bottles.

All of the 24 soil samples tested had ORP values in the range of +352 to +360 mV indicating that the soil samples were all moderately reduced. These ORP values were probably caused by the soil containing less organic carbon than the sludge sediment and that this available organic material was oxidized upon storage.

TABLE 7: RESULTS OF VITAL DYE TESTING

	Ascaris		Toxocara		Trichuris		Hymenolepis	
	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive
Trypan Blue	-	-	-	-	-	-	-	-
Nile Blue Sulfate	+	-	-	+	-	-	-	-
Methylene Blue	+	+	+	+	+	+	+	+
Methyl Violet	+	+	+	+	+	+	+	+
Crystal Violet	+	-	+	-	+	+	+	+
Janus Green	-	-	-	-	-	-	-	-
Brilliant Red	-	-	-	-	-	-	-	-
Benzopurpurin	-	-	+	-	+	-	-	-
Saphranin O	+	+	+	+	+	-	+	-
Carmin Alum Lake	+	+	-	-	-	-	-	-
Br. Cresyl Blue	+	+	+	+	+	+	+	-
Neutral Red	+	+	+	+	+	-	+	-
NILE BLUE SULFATE FRACTIONS:								
Acetone	-	-	+	+	-	-	-	-
Chloroform	-	-	-	-	-	-	-	-
Methanol	+	+	+	+	+	-	+	+
Ethanol	+	+	+	+	+	-	+	+
Acetone:ethanol	+	+	+	+	+	-	+	+

+ = dye incorporated into the egg

- = dye excluded by the egg

OTHER COMMENTS

Initially it was hoped that the soil samples would be a suitable control for the comparison of the inactivation of the test parasite eggs in the digested sludges. Unfortunately, recovery of the test parasite eggs were generally less from the soil samples. As suggested earlier, other types of controls may have been more suitable, but further studies are needed.

The standard deviations for the recovery and viability of the test parasite eggs points to the need for further investigation on improving the analytical efficiencies. Small physical changes in sludge characteristics, pH, particle charges etc. may all play a significant role in affecting the efficiency of the recovery of parasite eggs from sludges.

REFERENCES

1. Carroll, E.E., D.L. Masse, J.M. Genco and C.N. Ifeadi. Review of Land-Spreading of Liquid Municipal Sewage Sludge. EPA - 670/2-75-049, U.S. Environmental Technology Series, 1975.

2. Bond, J.D. The Risk of Ascaris Infestation From the Use of Human Sludge as Lawn Fertilizer. J. Fla. Med. Assoc., 44:964-967, 1958.

3. Marti, O.G., C.V. Booram and O.M. Hale. Survival of Eggs and Larvae of Swine Nematode Parasites in Aerobic and Anaerobic Water Treatment Systems. J. Environ. Qual., 9:401-405.

4. Theis, J.H., V. Bolton and D.R. Storm. Helminth Ova in Soil and Sludge From Twelve U.S. Urban Areas, J. Water Poll. Cont. Fed., 50:2485-2493, 1978.

5. Fitzgerald, P.R. Helminth and Heavy Metals Transmission From Anaerobically Digested Sewage Sludge. EPA - 600/S2-81-024, U.S. Environmental Protection Agency Project Summary, Cincinnati, Ohio, 1981.

6. Stern, G. and J.B. Farrell. Sludge Disinfection Techniques. In: Proceedings of the 1977 National Conference on Composting of Municipal Residues and Sludges, Information Transfer, Inc., Rockville, Maryland, 1977, pp. 142-148.

7. Process Design Manual for Sludge Treatment and Disposal. EPA 625/1-79-011, U.S. Environmental Protection Agency, 1979, Section 7-10.

8. USEPA. Agricultural Benefits and Environmental Changes Resulting From the Use of Digested Sludges on Field Crops. Interim Report on a Solid Waste Demonstration Project - Report SW-30d, 1971.

9. Sacramento Area Consultants. Sewage Sludge Management Program Final Report, Volume 6, Miscellaneous Use Determinations. Sacramento Regional County Sanitation District, Sacramento, California, 1979.

10. Schatzle, M. Investigations on the Effects of Sewage Sludge From Oxidation Channels on the Viability of Worm Eggs. Z. Wass. Abwass. Forsch., 2:147-150, 1969.

11. Veerannan, K.M. Some Experimental Evidence on the Viability of Ascaris lumbricoides ova. Current Sci., 46: 386-397, 1977.

12. Reimers, R.S., M.D. Little, A.J. Englande, D.B. Leftwich, D.D. Bowman, and R.F. Wilkinson. Parasites in Southern Sludges and Disinfection by Standard Sludge Treatment, EPA - 600/S2-81-066, U.S. Environmental Protection Agency Project Summary and Manuscript, Cincinnati, Ohio, 1981.

13. Hundley, D.F., and A.K. Berntzen. Collection, Sterilization and Storage of Hymenolepis diminuta Eggs. J. Parasitol., 55:1095-1096, 1969.

14. Black, M.I., P.V. Scarpino, C.J. O'Donnell, K.B. Meyer, J.V. Jones, E.S. Kaneshiro. Survival Rates of Parasite Eggs in Sludge During Aerobic and Anaerobic Digestion. J. Appl. Environ. Microbiol., 44:1138-1141, 1982.

15. Fitzgerald, P., and R. Ashley. Differential Survival of Ascaris Ova in Wastewater Sludge. J. Water Poll. Cont. Fed., 49:1722-1724, 1977.

16. MacInnis, A.J., and M. Voge. Experiments and Techniques in Parasitology. W.H. Freeman and Company, San Francisco, CA, 1970. p. 133.

17. Swartzwelder, J.C., J.H. Muller, S.H. Abadie, and L.G. Warren. Helminths. In: Manual for Clinical Microbiology, J.F. Blain, E.G. Lennette, and J.P. Traut, eds, American Society for Microbiology, Bethesda, MD, 1970. pp. 415-431.

18. von Brand, T., P.P. Weinstein, B. Mehlman, and E.C. Weinbach. Observations on the Metabolism of Bacteria-Free Larvae of Trichinella spinalis. Exptl. Parasitol. 1:245-255, 1952.

19. Patrick, W.H., Jr., and I.C. Mahapatra. Transformations and Availability of Nitrogen and Phosphorus in Waterlogged Soils. Advan. Argon. 20:323-359, 1968.

APPENDIX A

GEOMETRIC MEANS AND STANDARD DEVIATIONS

Tables A-1 through A-21 indicate for each month and test condition the geometric means and standard deviations for the specific test parasites either recovered or viable per 100 grams of sample weight. The solid content of soil and sludges were: 3% for soil, 2.5% for aerobic digested sludge and 3.2% for anaerobic digested sludge. To determine the number of parasite eggs either recovered or viable per gram of dry solids, divide the geometric mean by the solid content in the 100 gram wet weight sample.

For month 0, the usual number of samples analyzed for each test condition was three. When no samples were analyzed for a given test condition, the month 0 level was estimated by either using the initial amount of parasite eggs added or by averaging the measured levels of available data. For all other months, the usual number of samples analyzed for each test condition was eight, though as many as 20 samples were analyzed for a select number of soils after 33 months storage.

TABLE A-1: NUMBER OF ASCARIS EGGS RECOVERED PER 100 GRAMS WET WEIGHT

STORAGE CONDITION: 25° C

GEOMETRIC MEAN X / ÷ (S.D.)

Month	Soil	Aerobic (Phase I)	Aerobic (Phase II)	Anaerobic (Phase I)	Anaerobic (Phase II)
0	10,300.0 (1.1)	8300.0 (1.1)	4800.0 (1.0)	7800.0 (1.3)	5300.0 (1.1)
3	283.0 (2.0)	1.5 (26.5)	70.0 (2.0)	442.0 (1.8)	225.0 (2.9)
8	0.3 (6.3)	210.0 (5.5)	293.0 (2.1)	41.0 (1.5)	491.0 (6.2)
10	11.9 (8.1)	269.0 (1.3)	731.0 (1.5)	939.0 (4.2)	553.0 (2.9)
13	3.0 (7.3)	863.0 (1.6)	232.0 (2.3)	1407.0 (1.5)	1414.0 (1.3)
16	6.2 (15.1)	268.0 (1.6)	52.0 (1.8)	3367.0 (1.2)	45.0 (4.8)
19	4.1 (5.6)	102.0 (1.1)	84.9 (3.0)	77.1 (6.1)	645.0 (1.9)
22	14.1 (3.4)	7.5 (3.0)	526.0 (1.6)	154.0 (7.8)	186.0 (1.7)
25	30.7 (4.0)	328.0 (2.9)	266.0 (2.1)	384.0 (2.5)	410.0 (1.7)
30	5.4 (7.4)	7.1 (2.2)	38.2 (4.3)	a	a
33	14.3 (2.1)	48.3 (5.3)	10.7 (8.6)	a	a

a. No analyses conducted

TABLE A-2: NUMBER OF VIABLE ASCARIS EGGS PER 100 GRAMS WET WEIGHT

STORAGE CONDITION: 25° C

GEOMETRIC MEAN X / ÷ (S.D.)

Month	Soil	Aerobic (Phase I)	Aerobic (Phase II)	Anaerobic (Phase I)	Anaerobic (Phase II)
0	7700.0 (1.1)	7700.0 (1.1)	4500.0 (1.0)	7400.0 (1.3)	5000.0 (1.1)
3	270.0 (2.0)	1.0 (27.7)	30.9 (3.7)	400.0 (1.8)	208.0 (1.6)
8	0.3 (5.7)	146.0 (6.2)	265.0 (2.1)	13.0 (28.7)	223.0 (5.3)
10	11.0 (7.7)	187.0 (1.4)	528.0 (1.6)	269.0 (4.5)	107.0 (4.4)
13	a	187.0 (1.5)	65.2 (3.1)	146.0 (1.2)	58.5 (1.7)
16	0.2 (2.7)	a	a	a	a
19	0.2 (2.7)	0.3 (5.2)	a	a	a
22	0.3 (6.6)	0.2 (4.4)	a	a	a
25	1.5 (16.1)	0.9 (9.0)	0.2 (3.4)	a	a
30	0.4 (7.8)	a	0.2 (3.1)	b	b
33	1.4 (7.3)	a	a	b	b

a. The geometric mean is 0.125 and the standard deviation is (1). To facilitate calculating geometric means, 0.125 was substituted for zero values measured.

b. No analyses conducted.

TABLE A-3: NUMBER OF *ASCARIS* EGGS RECOVERED PER 100 GRAMS WET WEIGHT

STORAGE CONDITION: IN-GROUND

GEOMETRIC MEAN X / ÷ (S.D.)

Month	Soil	Aerobic (Phase I)	Aerobic (Phase II)	Anaerobic (Phase I)	Anaerobic (Phase II)
0	10,300.0 (1.1)	8300.0 (1.1)	4800.0 (1.0)	7800.0 (1.3)	5300.0 (1.1)
3	117.0 (2.7)	381.0 (4.9)	504.0 (2.9)	1454.0 (1.3)	266.0 (3.7)
8	35.5 (4.5)	923.0 (3.4)	212.0 (8.2)	114.0 (17.1)	29.0 (6.0)
10	17.6 (3.5)	1127.0 (2.2)	383.0 (2.4)	223.0 (3.1)	626.0 (2.2)
13	3.2 (2.2)	503.0 (3.2)	169.0 (3.8)	1241.0 (2.4)	772.0 (1.9)
16	1.5 (6.3)	802.0 (1.8)	257.0 (1.6)	281.0 (2.4)	59.0 (2.6)
19	1.8 (6.1)	1185.0 (1.8)	124.0 (2.4)	109.0 (4.8)	206.0 (12.6)
22	11.0 (3.0)	890.0 (2.4)	610.0 (2.1)	2429.0 (1.2)	646.0 (5.5)
25	32.5 (3.1)	1412.0 (1.7)	342.0 (3.3)	1057.0 (2.7)	1594.0 (1.3)
30	28.6 (2.9)	194.0 (3.0)	64.7 (2.6)	a	224.0 (5.1)
33	22.5 (3.3)	128.0 (5.2)	934.0 (1.8)	a	570.0 (2.8)

a. No analyses conducted.

TABLE A-4: NUMBER OF VIABLE ASCARIS EGGS PER 100 GRAMS WET WEIGHT

STORAGE CONDITION: IN-GROUND

GEOMETRIC MEAN X / ÷ (S.D.)

Month	Soil	Aerobic (Phase I)	Aerobic (Phase II)	Anaerobic (Phase I)	Anaerobic (Phase II)
0	7700.0 (1.1)	7700.0 (1.1)	4500.0 (1.0)	7400.0 (1.3)	5000.0 (1.1)
3	112.0 (2.6)	299.0 (6.0)	455.0 (3.0)	1406.0 (1.3)	230.0 (1.1)
8	33.8 (4.5)	825.0 (3.5)	207.0 (8.1)	2.7 (33.0)	1.6 (17.8)
10	10.8 (7.6)	11011.0 (2.2)	356.0 (2.4)	8.3 (10.5)	48.1 (4.5)
13	0.8 (4.9)	432.0 (3.3)	159.0 (3.7)	260.0 (2.7)	202.0 (2.1)
16	0.5 (5.0)	596.0 (1.8)	161.0 (2.6)	b	0.2 (3.4)
19	b	504.0 (1.9)	0.2 (4.3)	b	266.0 (7.9)
22	0.6 (6.0)	225.0 (2.6)	187.0 (4.8)	0.3 (5.2)	6.3 (12.6)
25	0.5 (5.9)	283.0 (1.9)	119.0 (4.9)	0.3 (4.4)	3.0 (17.3)
30	3.2 (8.8)	1.0 (10.2)	a	b	0.7 (6.7)
33	2.7 (10.3)	a	a	b	a

a. The geometric mean is 0.125 and the standard deviation is (1). To facilitate calculating geometric means, 0.125 was substituted for zero values measured.

b. No analyses conducted.

TABLE A-5: NUMBER OF ASCARIS EGGS RECOVERED PER 100 GRAMS WET WEIGHT

STORAGE CONDITION: 4^{o} C

GEOMETRIC MEAN X / ÷ (S.D.)

Month	Soil	Aerobic (Phase I)	Aerobic (Phase II)	Anaerobic (Phase I)	Anaerobic (Phase II)
0	10,300.0 (1.1)	8300.0 (1.1)	4800.0 (1.0)	7800.0 (1.3)	5300.0 (1.1)
3	816.0 (2.7)	464.0 (6.3)	621.0 (1.5)	851.0 (5.6)	302.0 (5.4)
8	217.0 (3.2)	273.0 (3.8)	463.0 (2.4)	342.0 (3.7)	58.0 (7.9)
10	111.0 (4.6)	8.1 (5.7)	153.0 (1.5)	1.1 (10.1)	419.0 (2.8)
13	13.2 (10.3)	2028.0 (1.6)	257.0 (2.7)	1963.0 (2.0)	388.0 (5.4)
16	9.7 (7.0)	595.0 (2.3)	83.0 (1.5)	714.0 (3.5)	62.0 (4.8)
19	1.9 (11.8)	257.0 (3.7)	166.0 (15.6)	a	82.5 (26.1)
22	396.0 (3.0)	1109.0 (1.3)	1035.0 (1.8)	3264.0 (1.5)	289.0 (27.1)
25	261.0 (2.4)	1772.0 (1.4)	236.0 (4.7)	1574.0 (1.7)	177.0 (4.4)

a. Not available.

TABLE A-6: NUMBER OF VIABLE *ASCARIS* EGGS PER 100 GRAMS WET WEIGHT

STORAGE CONDITION: 4° C

GEOMETRIC MEAN X / ÷ (S.D.)

Month	Soil	Aerobic (Phase I)	Aerobic (Phase II)	Anaerobic (Phase I)	Anaerobic (Phase II)
0	7700.0 (1.1)	7700.0 (1.1)	4500.0 (1.0)	7400.0 (1.3)	5000.0 (1.1)
3	779.0 (2.7)	415.0 (7.1)	570.0 (1.6)	790.0 (5.9)	264.0 (5.7)
8	214.0 (3.2)	261.0 (3.8)	451.0 (2.5)	317.0 (3.8)	33.3 (5.8)
10	105.0 (4.6)	6.9 (5.5)	138.0 (1.5)	1.0 (9.1)	384.0 (3.2)
13	12.0 (9.5)	1872.0 (1.6)	228.0 (3.0)	1763.0 (2.2)	358.0 (5.4)
16	8.5 (7.0)	355.0 (3.5)	80.8 (1.5)	634.0 (3.8)	55.2 (5.5)
19	1.1 (12.5)	246.0 (3.8)	87.4 (42.9)	a	78.5 (25.4)
22	300.0 (2.7)	990.0 (1.3)	922.0 (1.9)	2771.0 (1.6)	250.0 (27.0)
25	166.0 (2.7)	672.0 (1.4)	195.0 (4.6)	1247.0 (1.7)	135.0 (5.0)

a. Not available.

TABLE A-7: NUMBER OF TOXOCARA EGGS RECOVERED PER 100 GRAMS WET WEIGHT

STORAGE CONDITION: 25° C

GEOMETRIC MEAN X / ÷ (S.D.)

Month	Soil	Aerobic (Phase I)	Aerobic (Phase II)	Anaerobic (Phase I)	Anaerobic (Phase II)
0	1205.0 (1.1)	1205.0 (1.1)	1310.0 (1.1)	1310.0 (1.0)	1175.0 (1.1)
3	463.0 (1.5)	2.3 (28.4)	217.0 (2.5)	600.0 (2.3)	302.0 (2.2)
8	1.1 (21.1)	327.0 (2.6)	423.0 (1.8)	71.4 (9.1)	341.0 (3.7)
10	21.2 (3.3)	401.0 (1.4)	545.0 (1.5)	632.0 (2.2)	406.0 (1.7)
13	21.1 (2.4)	500.0 (1.4)	216.0 (3.4)	661.0 (1.5)	746.0 (1.2)
16	31.1 (12.9)	152.0 (1.8)	82.5 (2.3)	962.0 (1.3)	36.1 (4.4)
19	37.0 (3.3)	69.8 (2.6)	62.5 (2.5)	73.0 (3.9)	224.0 (1.7)
22	16.0 (11.3)	199.0 (31.8)	376.0 (1.3)	56.2 (14.4)	90.3 (1.6)
25	58.8 (2.4)	115.0 (2.6)	145.0 (1.9)	183.0 (2.2)	264.0 (1.8)
30	6.9 (7.4)	5.9 (2.0)	40.8 (3.5)	a	a
33	27.2 (2.6)	78.1 (3.8)	35.1 (3.3)	a	a

a. No analyses conducted.

TABLE A-8: NUMBER OF VIABLE TOXOCARA EGGS PER 100 GRAMS WET WEIGHT

STORAGE CONDITION: 25° C

GEOMETRIC MEAN X / ÷ (S.D.)

Month	Soil	Aerobic (Phase I)	Aerobic (Phase II)	Anaerobic (Phase I)	Anaerobic (Phase II)
0	1205.0 (1.1)	1205.0 (1.1)	1180.0 (1.1)	1310.0 (1.0)	1030.0 (1.1)
3	419.0 (1.5)	2.2 (25.4)	116.0 (2.1)	532.0 (2.3)	182.0 (2.8)
8	1.1 (20.6)	a	a	a	0.4 (6.1)
10	18.6 (3.5)	a	a	a	a
13	20.9 (2.4)	a	a	a	a
16	18.0 (13.8)	0.2 (4.3)	a	0.2 (3.4)	a
19	36.4 (3.3)	a	a	a	a
22	16.0 (11.3)	a	a	a	a
25	58.4 (2.4)	0.2 (2.7)	a	a	a
30	6.7 (7.2)	a	a	b	b
33	21.4 (2.8)	a	a	b	b

a. The geometric mean is 0.125 and the standard deviation is (1). To facilitate calculating geometric means, 0.125 was substituted for zero values measured.

b. No analyses conducted.

TABLE A-9: NUMBER OF *TOXOCARA* EGGS RECOVERED PER 100 GRAMS WET WEIGHT

STORAGE CONDITION: IN-GROUND

GEOMETRIC MEAN X / ÷ (S.D.)

Month	Soil	Aerobic (Phase I)	Aerobic (Phase II)	Anaerobic (Phase I)	Anaerobic (Phase II)
0	1205.0 (1.1)	1205.0 (1.1)	1310.0 (1.1)	1310.0 (1.1)	1175.0 (1.1)
3	264.0 (1.7)	732.0 (1.7)	941.0 (1.3)	832.0 (1.7)	283.0 (2.2)
8	156.0 (1.5)	812.0 (2.9)	240.0 (28.7)	194.0 (4.7)	12.8 (23.3)
10	72.1 (2.6)	864.0 (1.2)	633.0 (1.5)	532.0 (1.5)	318.0 (1.9)
13	43.1 (2.6)	529.0 (1.6)	396.0 (3.1)	398.0 (2.1)	655.0 (1.3)
16	9.2 (6.6)	522.0 (1.7)	342.0 (1.6)	233.0 (2.3)	36.9 (2.1)
19	1.7 (10.4)	471.0 (2.2)	120.0 (1.6)	121.0 (2.3)	195.0 (7.0)
22	20.8 (4.4)	361.0 (2.3)	401.0 (1.6)	776.0 (1.2)	175.0 (8.0)
25	34.3 (3.6)	546.0 (1.7)	120.0 (5.8)	710.0 (1.3)	509.0 (1.3)
30	49.3 (2.5)	398.0 (1.6)	94.8 (2.5)	a	266.0 (1.5)
33	38.4 (3.3)	209.0 (3.0)	396.0 (1.8)	a	232.0 (3.8)

a. No analyses conducted.

TABLE A-10: NUMBER OF VIABLE TOXOCARA EGGS PER 100 GRAMS WET WEIGHT

STORAGE CONDITION: IN-GROUND

GEOMETRIC MEAN X / ÷ (S.D.)

Month	Soil	Aerobic (Phase I)	Aerobic (Phase II)	Anaerobic (Phase I)	Anaerobic (Phase II)
0	1205.0 (1.1)	1205.0 (1.1)	1180.0 (1.1)	1310.0 (1.0)	1030.0 (1.1)
3	237.0 (1.7)	603.0 (1.8)	873.0 (1.3)	736.0 (1.6)	195.0 (2.5)
8	131.0 (1.6)	26.2 (11.8)	368.0 (16.7)	0.3 (5.2)	0.2 (6.6)
10	55.2 (2.6)	30.0 (1.7)	11.9 (2.5)	a	0.2 (2.7)
13	36.7 (2.8)	1.9 (5.7)	0.4 (9.5)	0.2 (5.3)	a
16	8.6 (6.5)	0.2 (3.4)	a	a	a
19	1.7 (10.4)	a	a	a	a
22	19.9 (4.4)	a	a	a	a
25	30.0 (4.2)	a	a	a	a
30	46.7 (2.4)	a	a	b	a
33	29.6 (3.0)	a	a	b	a

a. The geometric mean is 0.125 and the standard deviation is (1). To facilitate calculating geometric means, 0.125 was substituted for zero values measured.

b. No analyses conducted.

TABLE A-11: NUMBER OF TOXOCARA EGGS RECOVERED PER 100 GRAMS WET WEIGHT

STORAGE CONDITION: 4° C

GEOMETRIC MEAN X / ÷ (S.D.)

Month	Soil	Aerobic (Phase I)	Aerobic (Phase II)	Anaerobic (Phase I)	Anaerobic (Phase II)
0	1205.0 (1.1)	1205.0 (1.1)	1310.0 (1.1)	1310.0 (1.1)	1175.0 (1.1)
3	301.0 (3.3)	530.0 (6.4)	961.0 (1.2)	753.0 (2.0)	268.0 (2.5)
8	266.0 (2.7)	511.0 (1.5)	694.0 (1.4)	442.0 (2.1)	110.0 (6.0)
10	298.0 (4.3)	9.0 (17.4)	619.0 (1.2)	3.1 (16.5)	416.0 (1.6)
13	234.0 (3.4)	1096.0 (1.2)	622.0 (1.7)	878.0 (1.3)	405.0 (2.1)
16	82.9 (2.2)	531.0 (1.3)	279.0 (1.5)	320.0 (1.8)	98.3 (3.4)
19	41.6 (3.8)	159.0 (4.2)	398.0 (1.8)	a	235.0 (2.4)
22	1028.0 (1.8)	661.0 (1.2)	810.0 (1.3)	1014.0 (1.2)	124.0 (21.6)
25	403.0 (2.2)	660.0 (1.4)	414.0 (3.3)	654.0 (1.4)	164.0 (4.0)

a. Not available.

TABLE A-12: NUMBER OF VIABLE TOXOCARA EGGS PER 100 GRAMS WET WEIGHT

STORAGE CONDITION: 4^{O} C

GEOMETRIC MEAN X / ÷ (S.D.)

Month	Soil	Aerobic (Phase I)	Aerobic (Phase II)	Anaerobic (Phase I)	Anaerobic (Phase II)
0	1205.0 (1.1)	1205.0 (1.1)	1180.0 (1.1)	1310.0 (1.0)	1030.0 (1.1)
3	235.0 (3.1)	431.0 (7.0)	881.0 (1.1)	646.0 (2.3)	215.0 (2.9)
8	242.0 (2.7)	440.0 (1.5)	618.0 (1.4)	379.0 (2.2)	20.5 (20.4)
10	265.0 (4.3)	7.3 (15.4)	556.0 (1.1)	1.7 (17.7)	344.0 (1.7)
13	206.0 (3.5)	909.0 (1.2)	555.0 (1.7)	742.0 (1.4)	298.0 (2.2)
16	71.8 (2.3)	429.0 (1.3)	257.0 (1.5)	256.0 (1.8)	65.1 (3.9)
19	38.7 (3.6)	121.0 (4.9)	330.0 (1.8)	a	133.0 (2.7)
22	878.0 (1.9)	479.0 (1.2)	648.0 (1.2)	809.0 (1.1)	39.7 (35.5)
25	327.0 (2.2)	403.0 (1.5)	200.0 (4.8)	441.0 (1.4)	56.5 (4.1)

a. Not available.

TABLE A-13: NUMBER OF TRICHURIS EGGS RECOVERED PER 100 GRAMS WET WEIGHT

STORAGE CONDITION: 25° C

GEOMETRIC MEAN X / ÷ (S.D.)

Month	Soil	Aerobic (Phase I)	Aerobic (Phase II)	Anaerobic (Phase I)	Anaerobic (Phase II)
0	559.0 (1.1)	380.0 (1.0)	360.0 (1.1)	780.0 (1.2)	913.0 (1.1)
3	2.6 (13.5)	0.4 (7.0)	0.3 (5.7)	12.1 (2.0)	1.2 (14.2)
8	0.2 (2.1)	1.5 (8.3)	0.4 (8.5)	0.4 (5.0)	3.4 (16.8)
10	0.3 (5.7)	0.8 (5.3)	4.2 (4.9)	12.7 (17.7)	9.7 (6.6)
13	a	0.4 (6.9)	0.3 (6.6)	46.0 (1.7)	17.8 (7.6)
16	a	0.4 (7.4)	0.2 (4.4)	117.0 (1.6)	0.6 (8.1)
19	0.2 (2.7)	a	0.2 (9.9)	3.6 (4.4)	19.7 (3.0)
22	0.3 (8.0)	0.2 (4.4)	4.2 (4.3)	2.7 (7.3)	2.3 (6.5)
25	2.2 (6.9)	0.8 (7.1)	0.5 (7.6)	1.2 (6.9)	8.5 (6.5)
30	a	0.2 (2.7)	0.3 (4.4)	b	b
33	0.3 (4.6)	1.1 (8.2)	0.4 (7.5)	b	b

a. The geometric mean is 0.125 and the standard deviation is (1). To facilitate calculating geometric means, 0.125 was substituted for zero values measured.

b. No analyses conducted.

TABLE A-14: NUMBER OF VIABLE TRICHURIS EGGS PER 100 GRAMS WET WEIGHT

STORAGE CONDITION: 25° C

GEOMETRIC MEAN X / ÷ (S.D.)

Month	Soil	Aerobic (Phase I)	Aerobic (Phase II)	Anaerobic (Phase I)	Anaerobic (Phase II)
0	31.0 (1.1)	31.0 (1.0)	a	31.0 (1.0)	62.0 (1.0)
3	2.4 (12.3)	0.2 (3.2)	0.3 (5.7)	1.7 (9.4)	a
8	0.2 (2.1)	a	a	a	
10	0.3 (5.7)		0.2 (2.9)	a	a
13	a	a	a	0.3 (5.8)	a
16	a	a	a	a	a
19	a	a	a	a	a
22	0.3 (8.0)	a	a	a	a
25	0.8 (8.9)	a	a	a	a
30	a	a	a	b	b
33	a	a	a	b	b

a. The geometric mean is 0.125 and the standard deviation is (1). To facilitate calculating geometric means, 0.125 was substituted for zero values measured.

b. No analyses conducted.

TABLE A-15: NUMBER OF TRICHURIS EGGS RECOVERED PER 100 GRAMS WET WEIGHT

STORAGE CONDITION: IN-GROUND

GEOMETRIC MEAN X / ÷ (S.D.)

Month	Soil	Aerobic (Phase I)	Aerobic (Phase II)	Anaerobic (Phase I)	Anaerobic (Phase II)
0	559.0 (1.1)	380.0 (1.1)	360.0 (1.1)	780.0 (1.2)	913.0 (1.1)
3	0.4 (8.1)	10.9 (8.8)	15.1 (3.8)	25.8 (2.1)	0.4 (8.9)
8	1.1 (11.0)	4.9 (12.9)	0.3 (6.0)	0.3 (5.8)	a
10	2.4 (7.0)	12.0 (7.1)	6.1 (5.5)	3.9 (5.1)	24.4 (1.6)
13	0.2 (3.4)	6.3 (13.7)	1.2 (12.5)	35.4 (2.2)	8.0 (5.7)
16	a	10.1 (6.4)	0.3 (3.6)	1.6 (8.5)	0.3 (3.6)
19	1.4 (4.6)	22.1 (3.0)	3.0 (5.7)	3.6 (4.4)	2.5 (7.0)
22	0.4 (4.2)	5.4 (12.5)	0.9 (7.9)	50.2 (1.4)	25.0 (2.7)
25	0.5 (7.6)	13.6 (2.2)	2.8 (7.6)	55.5 (1.6)	21.9 (2.0)
30	6.3 (6.6)	1.1 (10.4)	0.5 (6.5)	b	1.5 (14.3)
33	0.6 (7.7)	1.7 (10.0)	18.0 (2.2)	b	16.5 (2.6)

a. The geometric mean is 0.125 and the standard deviation is (1). To facilitate calculating geometric means, 0.125 was substituted for zero values measured.

b. No analyses conducted.

TABLE A-16: NUMBER OF VIABLE TRICHURIS EGGS PER 100 GRAMS WET WEIGHT

STORAGE CONDITION: IN-GROUND

GEOMETRIC MEAN X / ÷ (S.D.)

Month	Soil	Aerobic (Phase I)	Aerobic (Phase II)	Anaerobic (Phase I)	Anaerobic (Phase II)
0	31.0 (1.0)	31.0 (1.0)	a	31.0 (1.0)	62.0 (1.0)
3	0.4 (6.8)	1.1 (22.3)	2.9 (19.9)	0.4 (9.1)	0.2 (5.2)
8	1.0 (10.5)	a	a	a	a
10	1.6 (5.7)	a	a	0.2 (3.9)	a
13	0.2 (3.4)	2.9 (15.9)	0.4 (8.8)	a	a
16	a	a	a	a	a
19	a	a	a	a	a
22	0.3 (3.6)	a	a	a	a
25	0.3 (4.0)	a	a	a	a
30	a	a	a	b	a
33	a	a	a	b	a

a. The geometric mean is 0.125 and the standard deviation is (1). To facilitate calculating geometric means, 0.125 was substituted for zero values measured.

b. No analyses conducted.

TABLE A-17: NUMBER OF TRICHURIS EGGS RECOVERED PER 100 GRAMS WET WEIGHT

STORAGE CONDITION: 4^{o} C

GEOMETRIC MEAN X / ÷ (S.D.)

Month	Soil	Aerobic (Phase I)	Aerobic (Phase II)	Anaerobic (Phase I)	Anaerobic (Phase II)
0	559.0 (1.1)	380.0 (1.0)	360.0 (1.1)	780.0 (1.2)	913.0 (1.1)
3	19.5 (4.1)	2.6 (13.0)	22.4 (1.7)	6.9 (35.6)	1.1 (38.8)
8	0.7 (10.9)	1.6 (8.8)	1.4 (12.8)	4.2 (6.3)	0.3 (6.0)
10	1.1 (16.2)	a	0.2 (3.1)	a	10.4 (2.4)
13	0.3 (5.2)	58.4 (1.9)	0.4 (6.8)	78.5 (1.9)	3.3 (16.1)
16	a	9.6 (6.9)	a	20.8 (11.8)	0.3 (4.9)
19	0.4 (4.2)	1.5 (8.7)	0.5 (6.8)	b	5.3 (3.9)
22	7.1 (15.6)	30.3 (2.1)	1.9 (13.8)	73.8 (2.1)	20.7 (2.0)
25	4.3 (11.2)	9.3 (1.9)	0.3 (5.5)	40.1 (1.9)	1.2 (12.9)

a. The geometric mean is 0.125 and the standard deviation is (1). To facilitate calculating geometric means, 0.125 was substituted for zero values measured.

b. No analyses conducted.

TABLE A-18: NUMBER OF VIABLE TRICHURIS EGGS PER 100 GRAMS WET WEIGHT

STORAGE CONDITION: 4° C

GEOMETRIC MEAN $\bar{X}$ / ÷ (S.D.)

Month	Soil	Aerobic (Phase I)	Aerobic (Phase II)	Anaerobic (Phase I)	Anaerobic (Phase II)
0	31.0 (1.0)	31.0 (1.0)	a	31.0 (1.0)	62.0 (1.0)
3	18.7 (3.9)	0.8 (12.3)	a	3.3 (34.2)	1.0 (33.4)
8	0.4 (9.3)	a	1.1 (10.2)	a	a
10	1.1 (15.1)	a	0.2 (3.1)	a	0.2 (3.1)
13	0.3 (5.2)	28.2 (2.0)	0.4 (6.8)	1.3 (11.9)	2.8 (13.8)
16	a	a	a	a	a
19	a	a	a	b	0.8 (12.2)
22	0.4 (9.3)	a	a	a	a
25	2.2 (11.3)	a	0.2 (3.1)	a	a

a. The geometric mean is 0.125 and the standard deviation is (1). To facilitate calculating geometric means, 0.125 was substituted for zero values measured.

b. Not available.

TABLE A-19: NUMBER OF HYMENOLEPIS EGGS RECOVERED PER 100 GRAMS WET WEIGHT

STORAGE CONDITION: 25° C

GEOMETRIC MEAN X / ÷ (S.D.)

Month	Soil	Aerobic (Phase I)	Aerobic (Phase II)	Anaerobic (Phase I)	Anaerobic (Phase II)
0	12,500.0 (1.0)	5100.0 (1.0)	5100.0 (1.1)	7600.0 (1.1)	7600.0 (1.1)
3	1477.0 (1.4)	54.5 (19.5)	429.0 (3.2)	1288.0 (2.3)	697.0 (1.3)
8	7.5 (7.9)	608.0 (2.6)	735.0 (1.9)	109.0 (8.1)	738.0 (2.2)
10	36.6 (3.3)	1092.0 (1.5)	1256.0 (1.3)	832.0 (1.4)	635.0 (1.5)
13	5.6 (5.5)	994.0 (1.2)	709.0 (1.6)	554.0 (1.4)	968.0 (1.3)
16	7.5 (3.6)	313.0 (1.7)	96.3 (1.8)	828.0 (1.2)	195.0 (2.0)
19	0.4 (7.1)	106.0 (2.0)	69.7 (2.0)	134.0 (1.5)	174.0 (1.8)
22	3.1 (4.5)	26.0 (4.7)	512.0 (1.3)	128.0 (2.0)	76.8 (1.5)
25	0.6 (8.2)	165.0 (2.1)	219.0 (1.4)	130.0 (1.9)	175.0 (2.2)
30	1.8 (5.4)	17.8 (1.7)	86.2 (3.1)	a	a
33	9.5 (2.3)	93.7 (2.3)	66.9 (1.9)	a	a

a. No analyses conducted.

TABLE A-20: NUMBER OF HYMENOLEPIS EGGS RECOVERED PER 100 GRAMS WET WEIGHT

STORAGE CONDITION: IN-GROUND

GEOMETRIC MEAN X / ÷ (S.D.)

Month	Soil	Aerobic (Phase I)	Aerobic (Phase II)	Anaerobic (Phase I)	Anaerobic (Phase II)
0	12,500.0 (1.0)	5100.0 (1.1)	5100.0 (1.1)	7600.0 (1.1)	7600.0 (1.1)
3	886.0 (1.6)	2371.0 (2.0)	2821.0 (1.3)	2758.0 (1.8)	1383.0 (1.9)
8	235.0 (2.3)	2049.0 (2.6)	685.0 (7.8)	187.0 (2.1)	165.0 (2.5)
10	115.0 (2.5)	2552.0 (1.2)	1646.0 (1.7)	472.0 (1.3)	69.0 (5.0)
13	47.4 (5.3)	1536.0 (1.5)	1504.0 (2.1)	103.0 (3.8)	1046.0 (1.3)
16	7.5 (6.5)	1568.0 (1.7)	780.0 (1.8)	206.0 (2.3)	54.5 (3.4)
19	0.7 (10.5)	1213.0 (1.3)	225.0 (2.4)	85.8 (2.0)	548.0 (2.4)
22	5.7 (5.4)	475.0 (4.9)	1004.0 (1.3)	431.0 (1.3)	63.1 (19.8)
25	10.8 (2.8)	659.0 (2.4)	272.0 (4.2)	415.0 (1.5)	246.0 (1.4)
30	5.3 (5.7)	281.0 (1.2)	214.0 (1.7)	a	258.0 (2.5)
33	10.7 (1.8)	245.0 (4.1)	560.0 (1.8)	a	213.0 (3.6)

a. No analyses conducted.

TABLE A-21: NUMBER OF HYMENOLEPIS EGGS RECOVERED PER 100 GRAMS WET WEIGHT

STORAGE CONDITION: 4° C

GEOMETRIC MEAN $\times / \div$ (S.D.)

Month	Soil	Aerobic (Phase I)	Aerobic (Phase II)	Anaerobic (Phase I)	Anaerobic (Phase II)
0	12,500.0 (1.0)	5100.0 (1.1)	5100.0 (1.1)	7600.0 (1.1)	7600.0 (1.1)
3	1773.0 (2.2)	2544.0 (3.5)	2609.0 (1.2)	2930.0 (2.0)	2158.0 (1.6)
8	1371.0 (2.6)	1593.0 (1.7)	2282.0 (1.3)	3480.0 (1.8)	1445.0 (2.2)
10	976.0 (3.6)	124.0 (3.9)	1821.0 (1.2)	843.0 (1.5)	2641.0 (1.4)
13	816.0 (2.4)	4190.0 (1.2)	2128.0 (1.5)	5080.0 (1.3)	3153.0 (1.3)
16	238.0 (1.9)	2169.0 (1.5)	973.0 (1.5)	2527.0 (1.5)	1658.0 (1.3)
19	78.9 (3.3)	763.0 (3.3)	886.0 (2.7)	a	917.0 (1.6)
22	1451.0 (1.9)	2136.0 (1.4)	1512.0 (2.0)	3121.0 (1.6)	734.0 (2.8)

a. Not available.

APPENDIX B

RESULTS OF ORP STUDY

The ORP values are related to the reference electrode used, in this case an Hg, Hg_2, Cl_2, saturated KCl electrode.

A. Anaerobic Phase I Sludge Stored at 25°C

sample	ORP
1	+406mV
2	+402mV
3	+296mV
4	+388mV
5	+390mV
6	+412mV
7	+402mV
8	+394mV

Approximately half of these samples are oxidized (characterized by an ORP greater than 400mV) and half are moderately reduced (indicated by an ORP of +400 to +100mV).

B. Anaerobic Phase I Sludge Stored at 4°C

sample	ORP
1	+418mV
2	+410mV
3	+420mV
4	+400mV
5	+410mV
6	+410mV
7	+410mV
8	+410mV

All of these samples are oxidized.

C. Anaerobic Phase I Sludge Stored in the ground

sample	ORP
1	+405mV
2	+393mV
3	+384mV
4	+398mV
5	+402mV
6	+382mV
7	+402mV
8	+398mV

The Orp values indicate that these samples are oxidized to moderately reduced sludge.

D. Aerobic Phase I Sludge Stored at 25°C

sample	ORP
1	+418mV
2	+412mV
3	+404mV
4	+410mV
5	+418mV
6	+412mV
7	+410mV
8	+418mV

All of these sludge samples are oxidized.

E. Aerobic Phase I Sludge Stored at 4°C

sample	ORP
1	+435mV
2	+430mV
3	+430mV
4	+410mV
5	+420mV
6	+400mV
7	+400mV
8	+410mV

All of these samples are oxidized.

F. Aerobic Phase I Sludge Stored in the ground

sample	ORP
1	+420mV
2	+418mV
3	+405mV
4	+420mV
5	+400mV
6	+420mV
7	+405mV
8	+420mV

All of these sludge samples oar oxidized.

G. Anaerobic Phase II Sludge Stored at 25°C

sample	ORP
1	+392mV
2	+384mV
3	+378mV
4	+376mV
5	+158mV (strong odor)
6	+408mV
7	+372mV
8	+390mV

Only one of the samples of sludge tested was in the oxidized state, all the rest were moderately reduced. Sample #5 was more reduced than the other samples and gave off a strong odor.

H. Anaerobic Phase II Sludge Stored at 4°C

sample	ORP
1	+362mV
2	+358mV
3	+356mV
4	+366mV
5	+360mV
6	+364mV
7	+366mV
8	+368mV

All of these sludge samples were moderately reduced.

I. Anaerobic Phase II Sludge Stored in the ground

sample	ORP
1	+370mV
2	+418mV
3	+382mV
4	+410mV
5	+364mV
6	+358mV
7	+362mV
8	+372mV

Two of these sludge samples were oxidized and the remainder were moderately reduced.

J. Aerobic Phase II Sludge Stored at 25°C

sample	ORP
1	+398mV
2	+400mV
3	+412mV
4	+415mV
5	+416mV
6	+412mV
7	+408mV
8	+416mV

All but one of the sludge samples were oxidized and that one was on the border between being oxidized and moderately reduced.

K. Aerobic Phase II Sludge Stored at 4°C

sample	ORP
1	+410mV
2	+420mV
3	+418mV
4	+405mV
5	+400mV
6	+350mV
7	+410mV
8	+400mV

Seven out of the eight sludge samples tested were oxidezed. The remaining sample was moderatelu reduced.

L. Aerobic Phase II Sludge Stored in the ground

sample	ORP
1	+400mV
2	+400mV
3	+390mV
4	+350mV
5	+360mV
6	+350mV
7	+380mV
8	+435mV

Three of the eight sludge samples analyzed were oxidized. The remaining five samples were moderately reduced.

M. Soil Stored at 25°C

sample	ORP
1	+384mV
2	+364mV
3	+372mV
4	+378mV
5	+382mV
6	+374mV
7	+392mV
8	+384mV

All of the soil control samples assayed were moderately reduced.

N. Soil Stored at 4°C

sample	ORP
1	+382mV
2	+378mV
3	+370mV
4	+374mV
5	+362mV
6	+378mV
7	+360mV
8	+388mV

All of the soil samples tested were moderately reduced.

O. Soil Stored in the ground

sample	ORP
1	+390mV
2	+388mV
3	+382mV
4	+384mV
5	+382mV
6	+374mV
7	+370mV
8	+368mV

All of the soil samples tested were moderately reduced.